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nature 451, 373-498 24 January 2008

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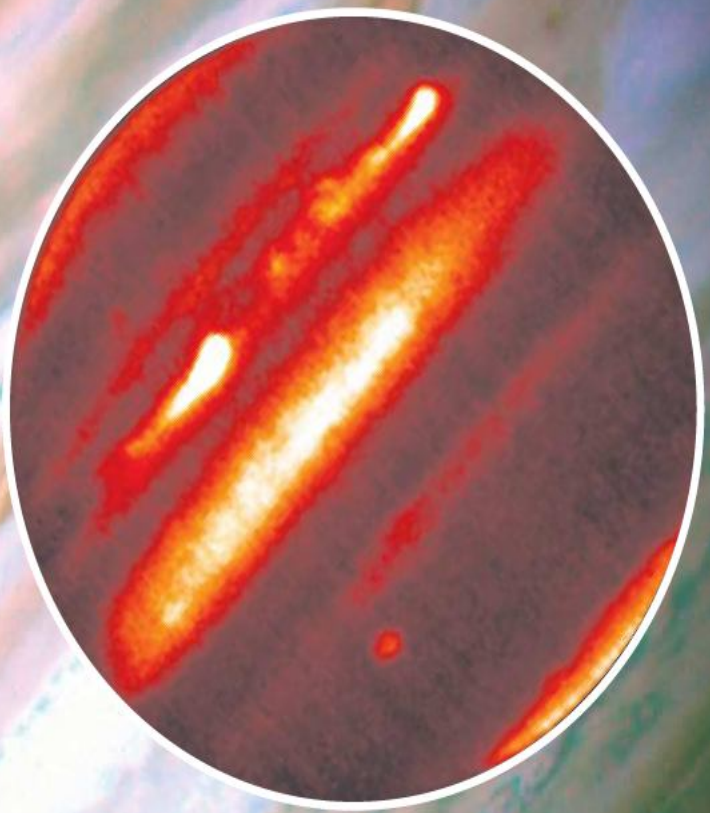
JUPITER'S FIERCEST JET

Storms probe a buried atmosphere



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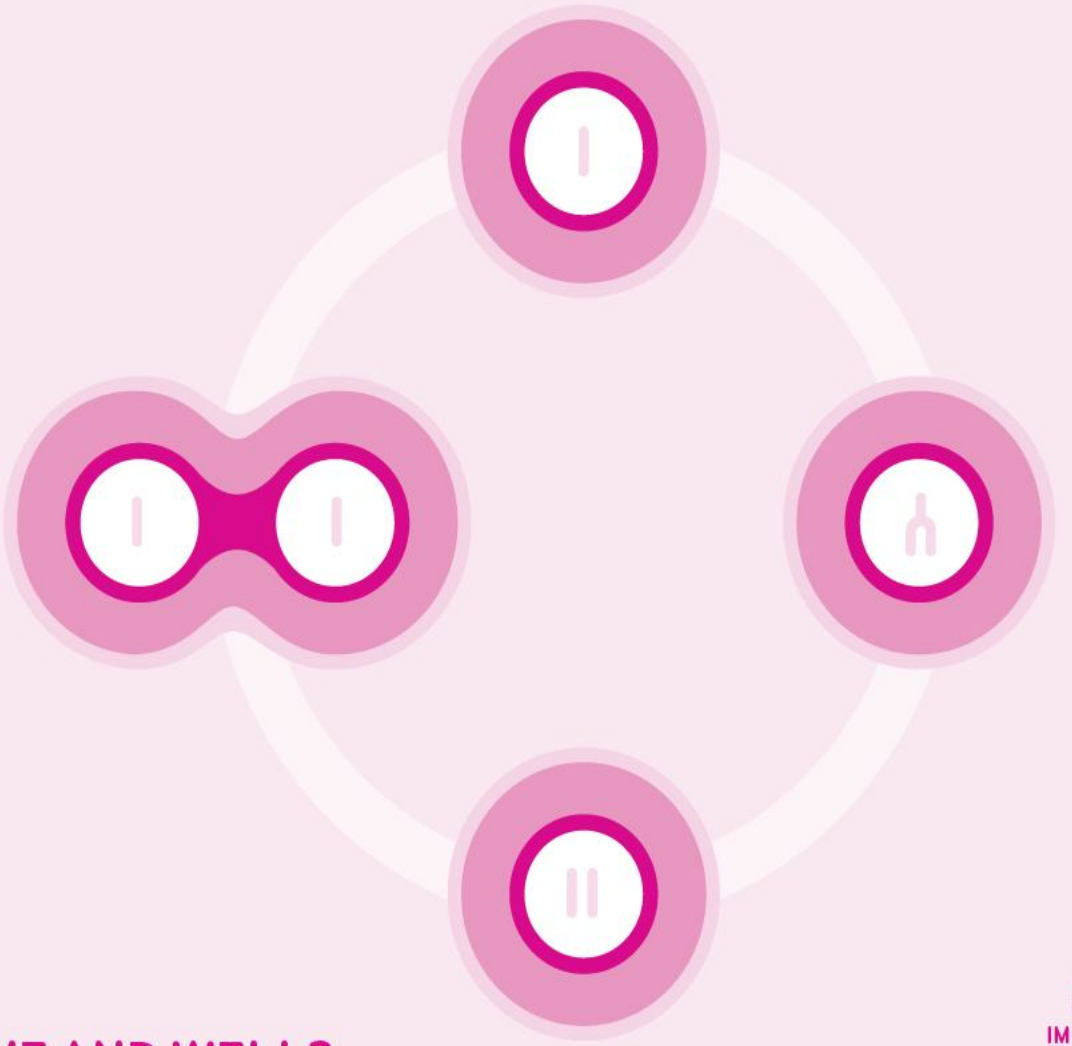

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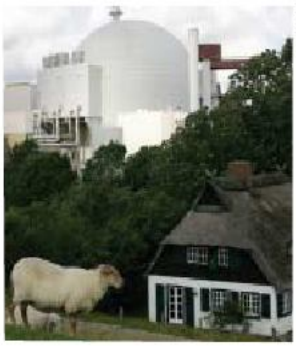
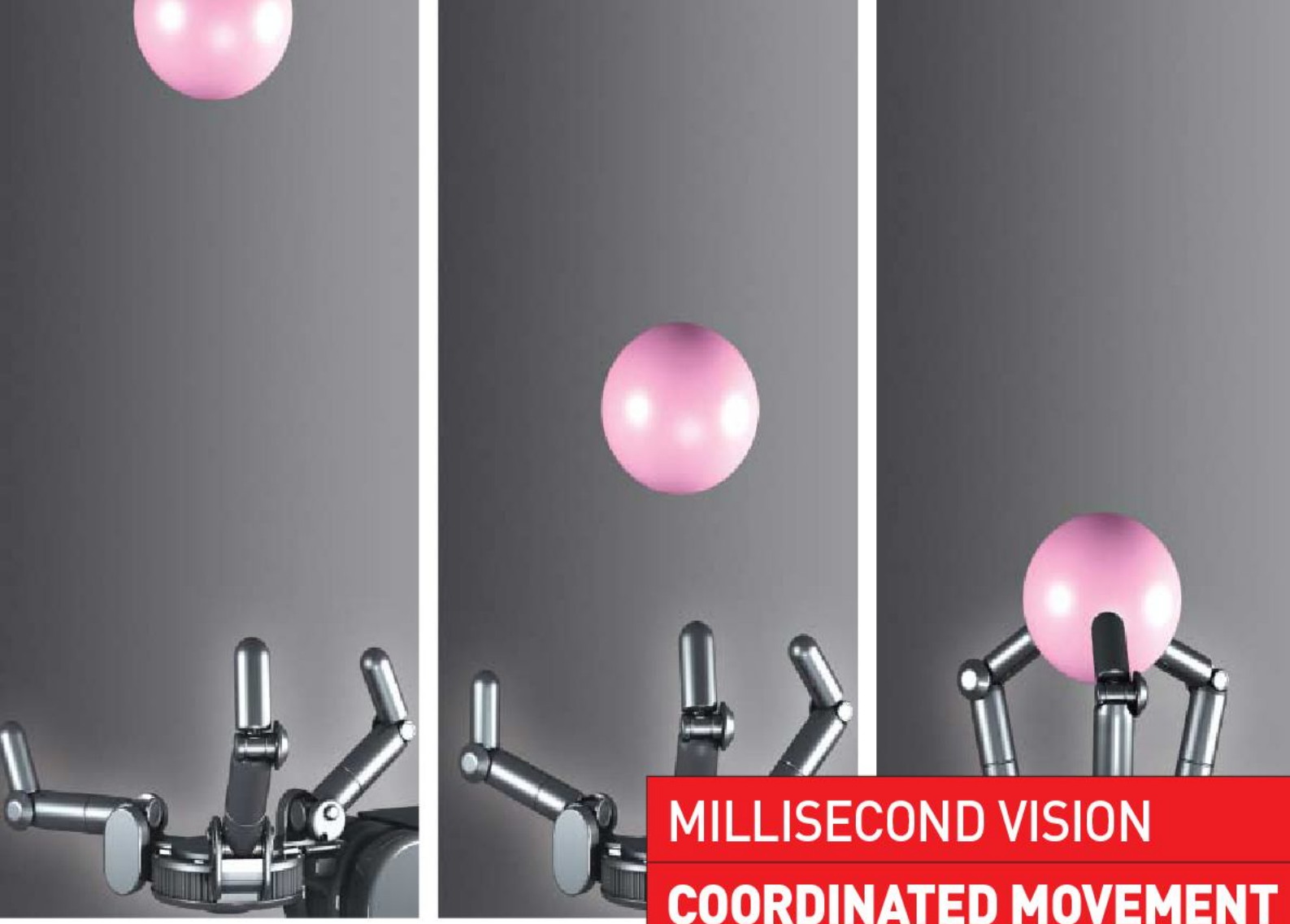
Collecting bug: natural history museums celebrated, p. **400**.


GETTY

May sheep safely graze? The hazards of nuclear power, p. **380**.



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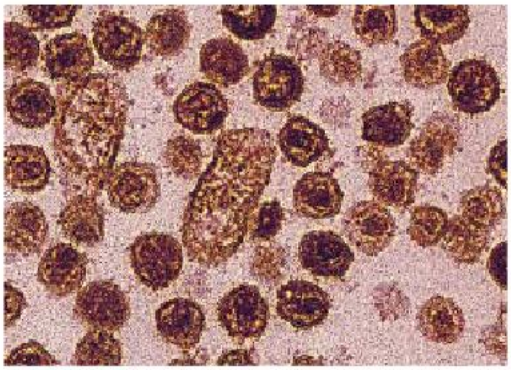
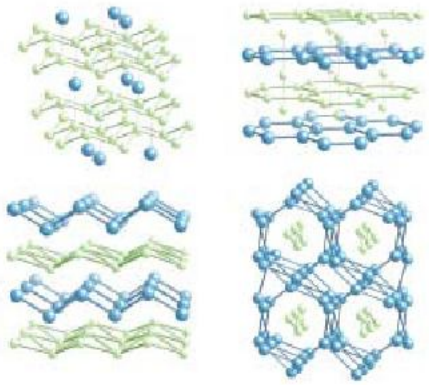


Holding on: how HIV neutralizes host-cell tetherin, pp. **425**, **406**.

REVIEW ARTICLE

ARTICLES

LETTERS



Lighten up: four possible lithium-beryllium alloys, p. **445**.

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Cell cycle control of centromeric repeat transcription and heterochromatin assembly
E S Chen, K Zhang, E Nicolas, H P Cam, M Zofall & S I S Grewal
doi:10.1038/nature06561

DARPA AT FIFTY

The late 1950s were busy, judging by the number of fiftieth anniversaries marked during 2007. One, the launch of Sputnik 1 and with it the space age, has its echo in 2008. DARPA, the US agency set up as a response to Soviet success in space, is 50 years old in a few weeks. This week's podcast, and features on pages 390 and 403, ask the questions, what has DARPA achieved, and what can it hope to achieve in the future?
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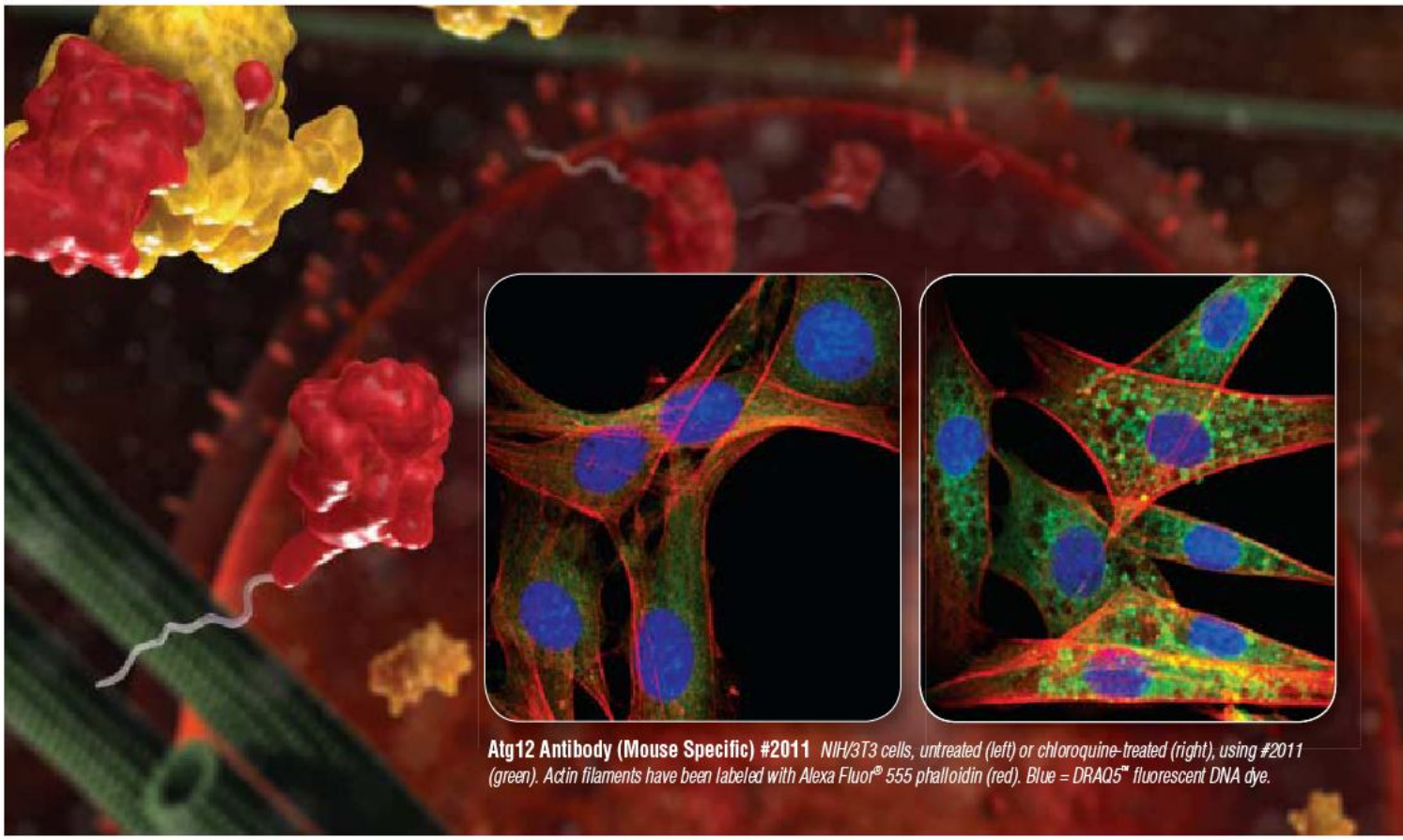

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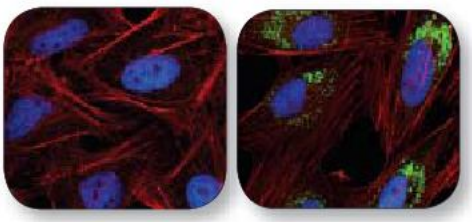

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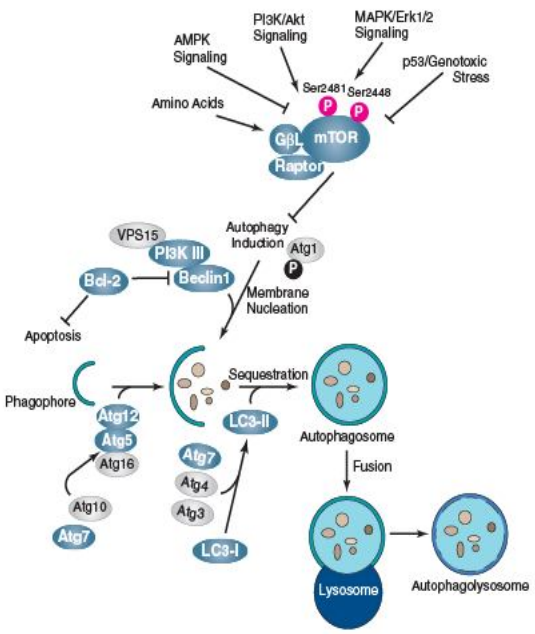

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THIS ISSUE

REPEAT OFFENDERS A literature trawl using the text-similarity search engine eTBLAST (http://tinyurl.com/2ex4ey) has uncovered a worrying trend: duplication, co-submission and plagiarism are on the increase. Mounir Errami and Harold Garner estimate the extent of the problem, and map out techniques that could be used to clean up our collective act. Chief among them, the convergence of journals' electronic submission systems with the new computational tools being developed to detect duplications. [Commentary p. **397**]

SMALL WONDERS Small RNAs, non-coding RNAs consisting of 20 to 30 nucleotides, regulate many biological processes. There may be tens of thousands of such regulatory RNAs in the mammalian genome. What do they all do? This Q&A session gets the general reader up to speed. [News & Views Q&A, p. **414**; www.nature.com/podcast]

NET GAINED DARPA, the Defense Advanced Research Projects Agency, was set up in 1958 as a response to the launch of Sputnik. DARPA was to ensure that the United States remained the most advanced military nation. As well as producing a military dividend, DARPA

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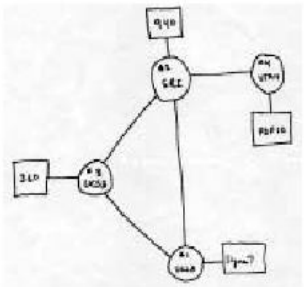

Internet ready: Arpanet, with four nodes, *circa* 1969.

technology has changed the commercial world: its Arpanet network paved the way for the Internet, and it helped create the Global Positioning System. To mark DARPA's fiftieth anniversary, we ask if it is still relevant today. [News Feature p. **390**] And former DARPA director Charles Herzfeld reflects on its successes and failures. [Essay p. **403**; www.nature.com/podcast]

ALTERNATIVE ENERGY Bacterial flagella contain a secretion apparatus related to the type III secretion system used by many pathogens to transfer effector proteins into host cells. It is generally assumed that the ATPase FliI provides energy for transport, but two reports argue against this view. Both groups find that flagellar secretion occurs even in the absence of the ATPase, and that the energy required derives from the proton motive force. [Letters pp. **485**, **489**]

HST/NASA-IRTF HAWAII

To coincide with the flyby of the Pluto-bound New Horizons probe, Jupiter was the target of intensive observation, starting in February 2007, from a battery of ground-based telescopes and the Hubble Space Telescope (HST). Weeks into the project, on 25 March, an intense disturbance developed in Jupiter's strongest jet at 23° North latitude, lasting to June 2007. This type of event is rare — the last ones were seen in 1990 and 1975. The onset of the disturbance was captured by the HST, and the development of two plumes was followed in unprecedented detail. The two plumes (bright white spots in the small infrared image on the cover) towered 30 km above the surrounding clouds. The nature of the power source for the jets that dominate the atmospheres of Jupiter and Saturn is a controversial matter, complicated by the interplay of local and planet-wide meteorological factors. The new observations are consistent with a wind extending deep into the atmosphere, well below the level reached by solar radiation. In the larger cover image, turbulence caused by the plumes can be seen in the band that is home to the jet. [Letter p. **437**; News & Views p. **409**]

Bonding exercise

It can be extremely difficult to alter a specific carbon–hydrogen bond in a complex molecule without modifying other parts of that molecule. But it can be done, and 'C–H activation' (or 'C–H functionalization') has received much attention recently, in part thanks to newly discovered catalysts that are able to selectively target and modify key C–H bonds in the presence of other functional groups. In a review, Huw Davies and James Manning explore how the insertion of metal carbenes and nitrenes into a specific C–H bond has been used to efficiently synthesize natural products and potential pharmaceutical agents. [Review Article p. **417**]

HIV/AIDS: ties that bind

Studies of Vpu, an HIV-1 accessory protein required for efficient HIV-1 particle release in some human cells, pointed to the existence of a tether based in a cell surface protein inducible by interferon-α. That tether has now been identified as the host cell molecule CD317—renamed tetherin — a membrane protein with no previously known function. Tetherin is shown to be involved in the retention of HIV-1 virions at the cell surface. Vpu neutralizes its effect, allowing the release and propagation of virus particles. Inhibition of Vpu function is therefore a possible therapeutic strategy in HIV/AIDS. [Article p. **425**; News & Views p. **406**]

Transposon watch

A large fraction of the eukaryotic genome consists of transposons, 'jumping genes' that move from place to place in the genome. They profoundly influence genome organization and have had a major impact on genome evolution. Host genomes have evolved mechanisms to control the spread of transposable elements, and one newly discovered mechanism is reported in this issue. It operates in fission yeast by exploiting the targeting of a specific class of transposons (*Tf2* retrotransposons, which act via RNA intermediates) by proteins of another class to rein in transposon activities. This seems to be an ancient retrotransposon surveillance pathway, and hints at conflict between DNA transposons and retrotransposons. [Article p. **431**; News & Views p. **412**]

Making light alloys work

The two lightest metallic elements, lithium and beryllium, do not interact with each other to form an alloy in ambient conditions. But the structure and also the reactivity of many compounds can be altered fundamentally by subjecting them to high pressure. A new computational study suggests that in the case of lithium and beryllium, four alloys — LiBe, $LiBe_2$, $LiBe_4$ and Li_3Be — should be stable at readily accessible pressures. Intriguingly, two-dimensional layers of almost ideal free-electron-like states appear within the three-dimensional crystal environment of one of the alloys, which may also have interesting superconducting properties. [Letter p. **445**]

Dirty ol' man river

The flow of dissolved inorganic carbon from rivers to the oceans is an important net flux connecting the terrestrial and marine carbon

LANDSAT

Patterns of change: Mississippi agriculture has left its mark.

reservoirs. Now a remarkable 100-year record of bicarbonate determinations, made at water treatment plants in the towns of Carrollton and Algiers, has been used as a basis for a study of Mississippi River water and carbon fluxes. Previous work revealed a significant increase the amount of dissolved inorganic carbon, mostly bicarbonate, exported by the Mississippi to the ocean over the past 50 years, but the cause for the increase remained uncertain. The Carrollton/Algiers data, together with sub-watershed and precipitation data, point to a mainly anthropogenic origin — increased bicarbonate discharge from agricultural watersheds that was not balanced by a rise in precipitation. [Letter p. **449**; News & Views p. **405**; www.nature.com/podcast]

The Hawiian connection

Hawaiian volcanoes are formed by the extrusion of large quantities of basaltic magma related to hot-spot activity below the Pacific plate. Despite many years of study, aspects of the topography characteristic of these volcanoes, such as rift zones, giant fault scarps and the resulting landslides, remain largely unexplained. Jean-Luc Got *et al.* use seismological data and finite-element mechanical modelling to show that surface topography may be controlled by the topography of the oceanic crust beneath the island, which is heavily deformed under the load of the volcano. [Letter p. **453**]

Subtle variation

It is natural to assume that patchiness in an ecosystem must reflect an underlying property of the habitat. Yet there are several lines of evidence to suggest that intrinsic biological dynamics can produce pattern even in an ecosystem that is homogeneous for the organisms involved. Outside of the laboratory, it is difficult to convincingly demonstrate large-scale pattern formation from biological interactions, mainly because it is close to impossible to exclude habitat variables. Vandermeer *et al.* have used the artificiality of shade trees planted in a coffee plantation to get around this problem, and demonstrate significant patterning of a particular species of ant, *Azteca instabilis*, that nests in those trees. Ant population density is controlled by natural enemies, mainly a parasitic fly, but the distribution of ant nest clusters follows a strong spatial pattern despite habitat homogeneity. [Letter p. **457**]

Stem cell renewal

Stem cell self-renewal involves the maintenance of pluripotency under continued proliferation. Proliferation of embryonic stem cells was thought to be constitutive and not to be regulated, because the normal cell cycle regulatory mechanism is not active in these cells. Now Andäng *et al.* present evidence for a fundamentally new mechanism of cell cycle regulation operating in embryonic stem cells and in other tissue-specific stem cell types. In it, endogenous GABA receptor signalling controls cell proliferation via a mechanism that involves cell cycle proteins previously associated with the cellular DNA damage checkpoint pathway. [Letter p. **460**]

Making connections

Downs syndrome cell adhesion molecules (Dscams) are adhesion molecules of the immunoglolulin superfamily. *Drosophila* Dscams have been implicated in the organization of neural connectivity, but little is known about the functions of the closely related molecules in vertebrates. Masahito Yamagata and Joshua Sanes now demonstrate a role for Dscam and DscamL in patterning of lamina-specific connections in the chick retina. Two other adhesion molecules, called Sidekick-1 and Sidekick-2, act in a similar way. These molecules are widely distributed in the nervous system and may be part of an 'adhesive code' that patterns neural connections in the brain. [Letter p. **465**] Further evidence for the importance of Dscams in vertebrate neural patterning comes from Fuerst *et al.*, who identify a role for DSCAM in establishing neural circuits in the retina of mice. [Letter p. **470**]

Gibberillins' light touch

Many developmental processes in plants are regulated jointly by light and by the gibberellins, yet the molecular basis of cross-talk between the two is not fully understood. Two groups now report results that reveal a signalling cascade that contributes to coordinated plant growth regulation by light and gibberellins. In the absence of gibberellins, DELLA proteins inhibit the binding of the transcription factor PIF3 (a phytochrome-interacting protein) to gene promoters. Gibberellins trigger the degradation of DELLA proteins, thereby allowing PIFs to bind to their target promoters and regulate gene expression. Light acts via a photoreceptor to destabilize PIF4. Thus the DELLA proteins, and competitive interactions between members of the PIF family, appear to key components linking light to the gibberellins. [Letters pp. **475**; **480**]

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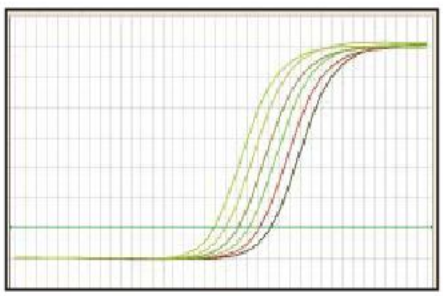

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Abstractions

LAST AUTHORS

Lithium and beryllium are the first metals encountered in the periodic table, but these light elements do not bond to one another — at least, not under ambient conditions. On page 445, renowned theoretical physicist Neil Ashcroft and Nobel-prize-winning chemist Roald Hoffmann, both at Cornell University in Ithaca, New York, report using computational methods to demonstrate that an alloy of these two metals, with unusual quantum behaviour, could be created at high pressures. They spoke to *Nature* about how bridging disciplines opens new frontiers.

Does chemistry have a role in quantum physics?

NA: The periodic table was created from an understanding of how elements react at ambient pressure. Now we need a greater understanding of how the chemistry, as well as quantum physics, of elements in combination changes under pressure.

RH: For me, this was a chance to convince physicists that chemists also understand bonding.

What initial findings led to this paper?

NA: No one thought a light alkali metal could ever be a superconductor until, in 1997, my group found that lithium could be at extremely low temperatures. Then we found that by putting lithium under high pressure, the superconducting temperature could be quite high. More recently, we wondered why beryllium, lithium's neighbour, is not a better superconductor. We decided to see whether forming a beryllium–lithium alloy improved its chances. Our computations suggest some of the alloys are stable at higher pressures, and they may even be good superconductors.

How might the alloy be useful?

RH: Unfortunately, it is not inherently useful — but then, neither was $E=mc^2$ at first. The world has a way of making new ideas useful. We gain insight into the nature of chemical bonds when we are able to make new compounds — in this case using pressure.

This paper was the work of one student and three professors. How did each of you contribute?

RH: We didn't know what structural combinations were possible between lithium and beryllium, so graduate student Ji Feng explored structures based on known chemical and physical rules while our colleague Richard Hennig searched random combinations for specific characteristics. Neil and I provided physical and chemical explanations for the findings — particularly the unusual two-dimensional electronic structures that emerged when modelled alloys were being squeezed in three dimensions. In the end, it was Ji that came up with the theoretical quantum mechanical model used in the paper to explain these structures. ■

MAKING THE PAPER

Paul Bieniasz

Unravelling a mechanism that stops HIV travelling between cells.

Retroviruses pose a major threat to the fitness and survival of many animals, including humans. Researchers have now identified a factor that keeps one type of the most notorious human retrovirus, HIV-1, stuck to the surface of human cells and unable to spread.

HIV-1 encodes a slew of proteins, and relies on host cells' machinery to make them. Among these is a set of accessory proteins that for some time didn't seem to be important for virus replication. One of these, Vpu, allows the virus to escape from the external surface of cells. HIV-1 viruses that lack Vpu get stuck, and cannot leave cells to invade others.

Paul Bieniasz, a virologist at the Aaron Diamond AIDS Research Center and Laboratory of Retrovirology at The Rockefeller University in New York, and his group began scouring the literature for cell-surface proteins that might hold on to HIV-1 in the absence of Vpu. They thought that Vpu might function by antagonizing such a protein — which they dubbed tetherin — allowing the virus to escape.

The search led the team to an adhesion molecule known as ICAM-1. "We knew that tetherin made the cell surface sticky, so a good candidate for that would be a cell-adhesion molecule," says Bieniasz. Earlier research had shown that ICAM-1 could be incorporated into HIV particles.

Stuart Neil, then a postdoctoral researcher in Bieniasz's laboratory, was keen to get to work. He quickly cloned ICAM-1 and introduced it and HIV-1 to cells. ICAM-1 seemed to inhibit the release of the viral particles, an effect that the addition of Vpu could counteract.

But, says Bieniasz, when Neil, now a lecturer at King's College London, repeated the same experiment using *ICAM-1* DNA that had been more thoroughly purified, "the effect completely went away". Although initially disappointing, the results ultimately provided a key piece of information. When cells are transfected with 'dirty' DNA, they sometimes produce interferon and other cytokines as part of an immune response. Bieniasz and Neil realized that the protein they were looking for was not ICAM-1, but one that could be induced by interferon.

After a series of experiments failed to isolate the mystery protein, the researchers reluctantly shifted strategy. They decided to compare gene expression in various cell lines, treating some of them with interferon-α. But they were pessimistic: "We thought there would be too many candidates to weed through," says Bieniasz. Instead, the results were "quite stunning", with fewer than 10 candidate genes meeting their selection criteria (see page 425).

Once the gene-expression data were in hand, the story unfolded very quickly. The team identified a favourite candidate within an hour, and the protein a week later. "It was very satisfying," Bieniasz says.

There is still much to learn about tetherin, however. There are hints that the protein has broad antiviral activity, yet few viruses make Vpu, which suggests that they have some other way of skirting tetherin's stickiness. Bieniasz also wonders whether genetic variation in tetherin might affect susceptibility to HIV infection or other viral diseases. So there's plenty of work to keep him tethered to the lab bench. ■

FROM THE BLOGOSPHERE

Welcome to Indigenus, the latest member of the NPG blogosphere! It is the blog of the equally new portal *Nature India* (http://www.nature.com/nindia/), a one-stop site for information on Indian science.

In her inaugural post, Subhra Priyadarshini, editor of *Nature India*, asks Indigenus readers what content *Nature India* should provide (http://tinyurl.com/3ba74g). Among the replies, Bikash Mohanty writes that much published Indian research is repetition of the work done in "so called scientifically advanced countries". He thinks *Nature India* should highlight original Indian research.

Arun Kumar Chokkappa suggests *Nature India* lists and tracks the funding opportunities that exist in the region, which "would be an eye opener for so many budding scientists who can't afford to knock on the doors of NIH and Wellcome Trust". In addition, Chokkappa believes that *Nature India* could help advance careers by putting ambitious students in touch with established scientists.

Vinod Jyothikumar hopes that the *Nature India* portal will result in more international collaborators becoming interested in research in India. ■

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Vol 451 | Issue no. 7177 | 24 January 2008

Fuel's paradise

The utopian urge to separate the world's nuclear-fuel cycles from national strategic ambitions has merit.

In 1972, Abdul Qadeer Khan, a young Pakistani engineer working at a uranium-enrichment plant in Almelo in the Netherlands, was able to walk out of the lab with the blueprints for advanced gas uranium centrifuges. Khan went on to become the father of Pakistan's atomic bomb, and created a nuclear-weapons black market network, exposed in 2004, spanning from labs and offices in Dubai, Malaysia and South Africa to clients in North Korea, Libya and Iran.

It is difficult to conclude what is most shocking: the shoddy security of the civilian nuclear industry, the unscrupulous scientist-accomplices in Khan's network, or that US intelligence agencies allegedly withheld information for well over a decade to protect US–Pakistan relations.

But they all add up to a stark reminder that the diversion of material from the civil nuclear industry remains the easiest stepping stone to nuclear-weapons prowess. The preventative oversight of the civil fuel cycle cannot necessarily be provided by plant operators, inspectors or governments. There is no regulatory requirement for a nuclear plant to be secure and 'proliferation resistant'.

Where then should efforts be focused to make the civil nuclear industry more secure? The answer is both simple and worrying. It has never been easier for the unscrupulous to construct a weapon. The most urgent challenge is to prevent anyone acquiring or producing the couple-of-dozen kilograms of fissile material required.

There are two points of danger in the nuclear-fuel cycle. One is at its front end: the enrichment of uranium to provide the low-enriched fuel needed to operate a reactor, a technology that is readily diverted to make highly enriched uranium for weapons. The other is at the cycle's back end: the reprocessing of spent fuel, which produces plutonium. Reprocessing is not in vogue and is uneconomic, although its use could grow if demand for nuclear energy puts pressure on uranium resources. For now, the pressing focus should be on uranium-enrichment technologies.

This challenge is all the more acute given that many countries, including Egypt and Saudi Arabia, are now turning to or at least considering nuclear power for strategic reasons that often go beyond their claimed motivations of energy independence and combating climate change. The existing enrichment capacity of countries such as Brazil and Japan makes them virtual weapons states — they could arm in months if they so wished.

Iran has made much of its 'inalienable right' under the 1970 nuclear non-proliferation treaty (NPT) to develop uranium-enrichment technology. Its insistence that this is a purely civilian endeavour rings manifestly false to many expert ears, for good reason. Beyond the various lines of evidence that support this scepticism lies the more general question of why bother. After all, neither Iran nor any other country has in principle any need to create national enrichment facilities. The international market for enriched uranium for civil use is efficient and well served, and good models for multilateral control already exist. Multilaterally owned or operated fuel banks have been on the agenda since the detonation of the first nuclear bomb. The concept of multilateral enrichment facilities provided by existing technology owners is an idea whose time has come: we should embrace it (see page 380).

"The concept of multilateral enrichment facilities is an idea whose time has come."

Such a solution requires a reinterpretation of the terms of the nuclear compromise embedded in the NPT, which offers countries unlimited opportunities for civilian nuclear development in return for forswearing proliferation. If new national enrichment facilities continue to be allowed, any nation that fears international sanctions somewhere down the line would have good reason to develop such facilities regardless of whether it also had military ambitions for the technology, as a matter of energy security. A fuel bank set in some way 'above politics' might help allay such fears — but that carrot would probably not, in itself, be enough. A further necessary condition for success would be that the nuclear-weapon states must themselves keep their part of the disarmament bargain (see *Nature* **451**, 107; 2008).

In general, this journal believes that all nations should be allowed to develop scientific and technological capacities as they wish. There is no merit in developing nations sitting as passive consumers of the knowledge and know-how that the developed world already boasts. But some technologies are exceptional. ■

Competition and noise

Mitt Romney's pledge to plough $20 billion a year into energy research may signal an unseemly bidding war.

It was, some commentators said, the mother of all panders. When Republican presidential contender Mitt Romney told a Detroit audience that he would increase US federal funding in energy research, automotive technology and materials science fivefold, to a cool $20 billion a year, those inside the Beltway shrugged. In the Motor City, though, the idea had more play. Romney's upbeat, go-get-'em approach may have resonated with voters, and he duly won the Michigan primary the next day.

Romney is only one of half-a-dozen viable candidates for this November's presidential election, and his ambitious proposal is unlikely to be put into effect. It is nevertheless significant, because it points to how the United States may choose to respond to the danger of an economic recession that would spread to the country at large the woes already felt by many voters in Michigan.

President Bush and Congress are already scrambling to put together a stimulus package to stave off recession. Strategic responses — such

as Romney's research plan — are now likely to feature prominently in the election campaign. Murmurs about 'competitiveness' that have been around for years will gain new salience

Romney's rhetoric will resonate with many physical scientists and engineers. "We spend $30 billion a year in the National Institutes of Health, and we lead the world in health-care products. In defence, we spend even more. We lead the world in defence products," he told the Detroit Economic Club on 14 January. "Why not also invest in energy and fuel technology right here in Michigan?" These sentiments come straight from the songsheet of the American Physical Society and its allies, which have long been pursuing a more modest proposal to double the amount the federal government spends on research in the physical sciences and engineering. But Romney's pledge of $20 billion promises much more.

This isn't some hick candidate pulling numbers out of the air: a former chief executive of Bain & Company, the Boston management consultancy, Romney knows what $20 billion is. And precedents suggest that a specific, costed commitment such as this one — made in the heat of the primary campaign — will be hard to discard if the candidate who made it is actually elected.

Even so, there is something unreal about Romney's proposal. Notably, it was made as part of a speech outlining the fantasy that the US motor industry can bounce back from its current emaciated state. General Motors and Ford have had some three decades of notice from Toyota, Nissan and Honda that it is time to shape up, and have utterly squandered every opportunity to respond. Significantly, Romney allied his research pledge with a call for looser fuel-efficiency standards — the signature cause of the Detroit industry, which has repeatedly signed its own death warrant by resisting innovation at every turn.

Senator John McCain (Republican, Arizona), Romney's rival in Michigan, promised action on global warming and told voters that their old jobs aren't coming back — a message they probably didn't want to hear. There will clearly be political mileage in the sort of fixes Romney promised to Michigan last week. And as the United States' economic troubles deepen in this election year, there may be more snap prescriptions for industrial revival, some of them based on heavy federal investment in research and development.

"As the United States' economic troubles deepen, there may be more snap prescriptions for industrial revival."

That represents a promise and a threat for scientists and engineers whose disciplines stand to benefit. The promise is considerable. As US physical scientists have been arguing for years, their work can help bolster industrial competitiveness, directly and indirectly. Thankfully, there is an even broader consensus (now shared by Republican and Democrat presidential candidates) that energy research is under-funded.

But Romney's disingenuous $20-billion pledge should be treated with scepticism. It is almost inconceivable that Congress would deliver that kind of money. Romney feebly suggested that it be diverted from federal job-training programmes, but these are scantily funded, and sure to grow, not contract, if recession arrives. And as for the justification of a comeback for Detroit, that particular movie ended decades ago and there will be no sequel. ■

A little less Disneyland

DARPA should focus on its founding values.

It is the ultimate playground for academics with big dreams: $3 billion to do whatever you want, as long as it's in the interests of defending the United States. An open chequebook, and no peer review, has meant great success for the Pentagon's research wing, the 250-strong Defense Advanced Research Projects Agency (DARPA). Over the past half-century, it has been the place where the best and the brightest gather to crack some of the toughest nuts in the defence community: how to build radar-defying stealth aircraft; how to detect hostile missile launches; how to build the best satellite-navigation system so that soldiers can find their way. 'DARPA hard' is the phrase that's used — if it's not hard enough for DARPA to do, it's not worth the agency doing it.

DARPA's accomplishments are legendary, so much so that other areas of the government are hoping to mimic its success with their own DARPA spin-offs (see pages 390 and 403). This is not an easy task but not necessarily a bad idea; at the very least, some parts of the government not known for innovation might begin to think about technological issues from a creative and higher-risk standpoint. But what is the future of DARPA itself?

Its 50-year mark is as good a time as any to take stock. Like the rest of the United States, DARPA can no longer afford to operate under a cold-war mentality; the terrorist attacks of 11 September 2001 and their aftermath have dramatically restructured the country's national defence needs. DARPA stumbled badly in one of its first and most prominent responses to the attacks by setting up an invasive information-mining programme run by a man convicted of multiple felonies (later reversed on a technicality) relating to the Iran–Contra scandal of the 1980s.

Today, some of DARPA's activities remain firmly rooted in its cold-war research past, which led to the unmanned drones flying surveillance missions in Iraq. But, as was on show in a California Disneyland hotel last year, the agency's director, Anthony Tether, has also tried jazzing things up a bit, by sponsoring competitions such as the $2-million 'grand challenge' robot car race. Even his critics note that Tether has managed to draw younger researchers into an agency whose stalwart backers are growing greyer every year.

Such approaches may be useful in helping the government think outside of the box. But DARPA must be careful not to stray too far down that path. The agency's future will depend in large part on the actions of Tether's successor: the position of DARPA director is a powerful one, with the ability to select programme managers and dispense money as he or she sees fit.

The agency's next director must take the agency back to basics, or risk losing the edge that comes from the inspired patronage of risky research. Above all, DARPA needs to concentrate more on projects that could lead to long-term pay-offs in the fight against terrorism, in ways we cannot yet imagine. ■

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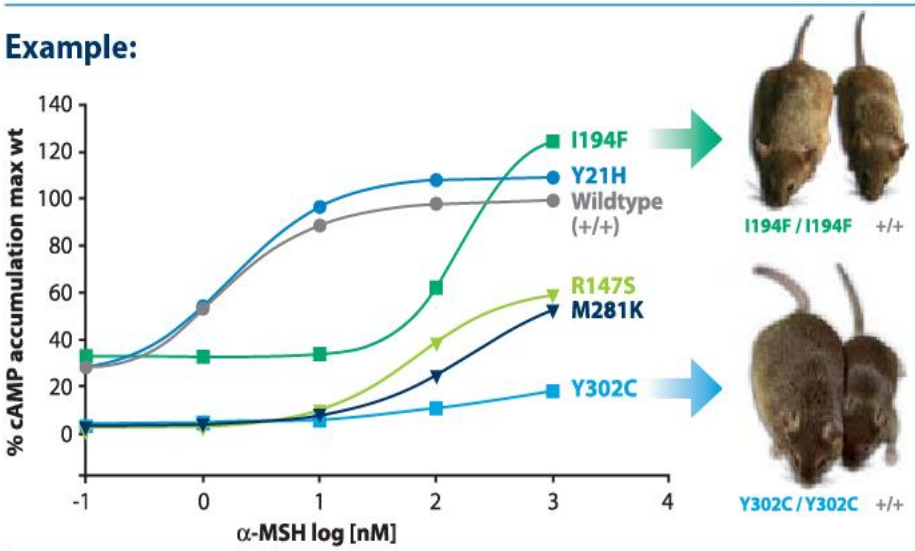

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RESEARCH HIGHLIGHTS

Leukaemia's origins

Science 319, 336–339 (2008)

Cells that cause the most common form of childhood cancer, acute lymphoblastic leukaemia (ALL), have been identified. They could serve as a target for therapy, or may be useful for monitoring the likelihood of relapse after treatment.

Children with ALL often harbour a small population of cells with a characteristic chromosomal rearrangement. Tariq Enver at the University of Oxford, UK, and his colleagues found that a specific subset of these cells cause a leukaemia-like disease when transplanted into mice with deficient immune systems.

The researchers also found similar cells in a healthy child whose identical twin sister has ALL. These cells seem to be immature versions of the cancer-causing ones, and could become malignant if triggered by additional mutations, the authors propose.

MURPHY FAMILY

GEOLOGY

Magnetic attraction

Earth Planet. Sci. Lett. doi:10.1016/j.epsl.2007.12.006 (2007)

A four-decade-old core, taken from the sea floor off the coast of Antarctica, is revealing new details about how Earth's magnetic field operates at high latitudes.

The US Navy Ship *Eltanin* collected the 16-metre-long core from the bed of the Ross Sea in 1968. Samples from the core then sat in storage until they were studied by a group of researchers led by Luigi Jovane of the University of California, Davis. They argue that its palaeomagnetic history is one of the most detailed ever recovered from either polar region.

For instance, the core records larger-than-expected variations in the high-latitude magnetic field. These can be explained by vortices generated far beneath the polar regions.

ARCHAEOLOGY

One-tonne rat

Proc. R. Soc. B doi:10.1098/rspb.2007.1645 (2008)

The skull of an enormous but extinct rodent has been found in a boulder on the coast of southern Uruguay by Andrés Rinderknecht of the country's Museum of Natural History and Anthropology, and Ernesto Blanco of the University of the Republic, both in Montevideo.

At 53 centimetres long, the skull's size suggests that its owner weighed more than 1,000 kilograms, making it the largest rodent on record. By comparison, the world's largest extant rodent, the capybara, tips the scales at about 60 kilograms.

The new species has been named *Josephoartigasia monesi*. The size of its teeth suggest it probably lived on soft vegetation and fruit, and on the basis of dating of the rocks in which it was found, the skull is thought to be 2 million to 4 million years old.

CONDENSED-MATTER THEORY

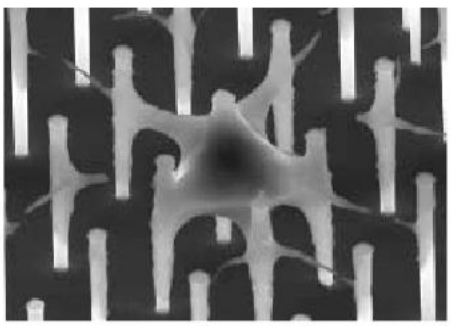
Drying drops

Europhys. Lett. 81, 26006 (2008)

One way to make an extremely waterproof material is to dapple a chemically hydrophobic surface with microscopic pillars on which beads of water can perch. Mathilde Reyssat, now of Harvard University, and her colleagues have done exactly that.

The authors sprinkled water onto the surface of this substance and allowed the drops to evaporate. Eventually, as the drops became smaller, they collapsed, running down between the posts (pictured below, side-on).

Reyssat and her team found some curious effects. As the water droplets shrank, for example, they tended to flatten before jumping back into a spherical shape.



ZOOLOGY

Do the dance

Proc. R. Soc. B doi:10.1098/rspb.2007.1620 (2007)

A honeybee colony led by a promiscuous queen does better than one led by a faithful queen: the colony forages more, stores more food and grows faster. Heather Mattila and her colleagues at Cornell University in Ithaca, New York, think this happens because genetically diverse colonies dance more.

Honeybees 'waggle dance' to tell each other where to fly to find food. Mattila's team compared colonies in which the queen always bred with the same male to colonies ruled by a queen that had been inseminated by 15 drones. On average, worker bees from the latter category performed 36% more dances daily, kept waggling for 62% longer and communicated about food discoveries farther from the nest than did workers from single-father colonies.

ORGANIC CHEMISTRY

Remote control

Angew. Chem. Int. Edn doi:10.1002/anie.200704963 (2008)

An organic reaction that is widely used in industry should become even more useful now that chemists in Japan have managed to control which of two mirror-image versions of its products are made.

Hydrocarbon fragments attached to a magnesium atom and a halogen atom are known as Grignard reagents, and are common building-blocks in reactions that generate carbon–carbon bonds. But a mixture of 'right'- and 'left'-handed

versions of the same molecular structure is usually produced, causing problems for the pharmaceutical industry, which requires drugs to be of one particular form.

Toshiro Harada and Yusuke Muramatsu of the Kyoto Institute of Technology reacted Grignard reagents with aldehydes in the presence of a titanium complex. This generated only one of the two possible versions of the product, in fewer steps than other methods.

NEUROSCIENCE

Toxic length

Neuron **57**, 27–40 (2008)

Huntington's disease, an inherited neurodegenerative disorder, is caused by genes that encode an abnormally elongated version of the protein huntingtin. Work by Juan Botas of Baylor College of Medicine in Houston, Texas, and his colleagues challenges the prevailing view that this protein does its damage only after it has been cleaved by enzymes and imported into the nucleus.

Botas and his co-workers transferred the expanded gene that describes the abnormal protein in humans into fruitflies. They observed toxic effects on the flies' brain cells before they could detect the protein inside those cells' nuclei.

They then pinned down the neurotoxicity to increased neurotransmitter release triggered by unusually high concentrations of calcium ions in certain brain cells. Tinkering with other genes that regulate neurotransmitter and calcium dynamics suppressed some of the mutant protein's deleterious effects, they add.

MATERIALS SCIENCE

His dark materials

Nano Lett. doi: 10.1021/nl072369t (2008)

A 'super-black' material has been made by researchers at the Rensselaer Polytechnic Institute in Troy, New York. It reflects just 0.045% of all the light of a certain wavelength and is therefore the darkest synthetic material ever, according to the group, led by Shawn-Yu Lin.

The substance is composed of row upon row of vertically aligned carbon nanotubes of different lengths, most of which have multiple walls. This arrangement gives the material a rough surface with no repeating topology, and causes it to scatter light in random directions, which is what makes it extremely black.

The material is also very dark across the rest of the visible spectrum, with a reflectance ranging from 0.045% to 0.07% as the wavelength changes from 633 nanometres (red) to 457 nanometres (blue). Such properties could prove useful in devices to capture solar energy.

PALAEOPHYSIOLOGY

T. rex teen pregnancies

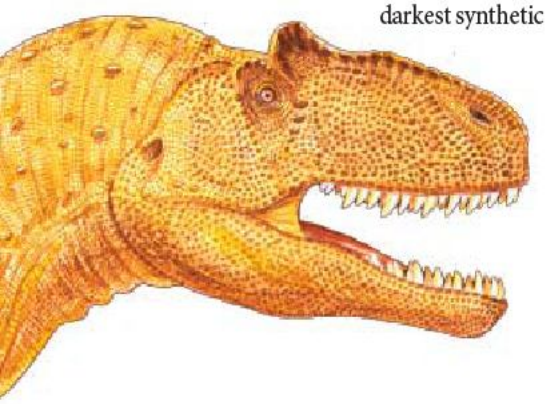
Proc. Natl. Acad. Sci. USA **105**, 582–587 (2008)

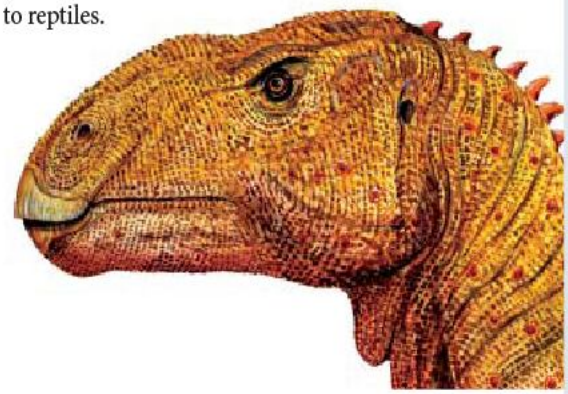
A new means of establishing sexual maturity in dinosaurs could finally settle the argument about whether they grew like big versions of modern lizards, or faster than such scaling would imply.

Andrew Lee, now at Ohio University in Athens, and Sarah Werning, of the University of California, Berkeley, have worked out the age by which two female dinosaurs had reached sexual maturity by looking at the specimens' medullary bones. Today, these short-lived tissues are found only in birds, where they provide calcium for developing egg shells, forming in the marrow cavities of females a few weeks before ovulation.

In 2005, another group reported medullary bones in a *Tyrannosaurus rex*, which was about 18 years old when it died. Lee and Werning's measurements of a *Tenontosaurus tilletti* (pictured right) and an *Allosaurus fragilis* (below) show that these species had reached maturity by 8 and 10 years of age, respectively. Had they grown like big lizards, these half-grown specimens would not have been fertile. The findings suggest that dinosaurs grew at rates more akin to today's birds and mammals than to reptiles.

B. KRZIC/NHMPL





JOURNAL CLUB

Hidde Ploegh
Whitehead Institute for Biomedical Research, Boston

An immunologist marvels at dendritic cells.

Dendritic cells ingest and process all manner of bacteria and viruses, and display the invaders' distinguishing structures so that other cells of the immune system 'know' what to 'look for'. No adaptive immune response can begin without them. For years I have been fascinated by the internal details of dendritic cells that enable them to handle this task.

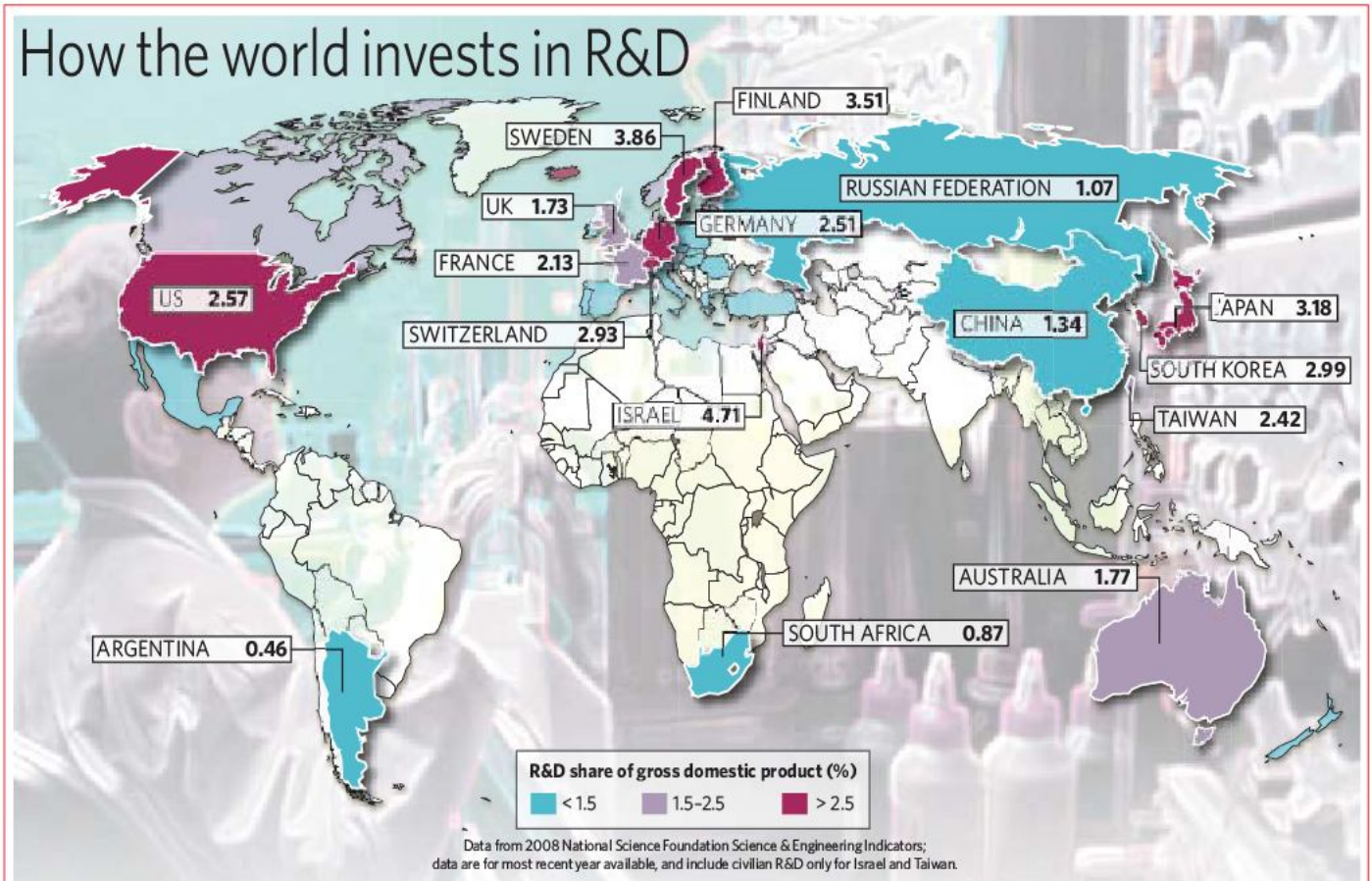
One set of details concerns how these cells manage protein production and disposal, given that breaking down and presenting sections of foreign proteins are the cells' primary jobs. Last month, Hugues Lelouard and his colleagues at the University of the Mediterranean in Marseille, France, discovered that dendritic cells fine-tune the translation of messenger RNAs to proteins when they are activated by inflammatory stimuli (H. Lelouard *et al. J. Cell Biol.* **179**, 1427–1439; 2007).

The authors' stimuli of choice were lipopolysaccharides, signature molecules that indicate the presence of certain bacteria. Dendritic cells exposed to them showed a close correlation between the extent to which translation became more efficient and the increased formation of lumpy bodies similar to aggresomes, which is a prelude to the destruction of proteins. The authors then elucidated the biochemical steps that lead to enhanced translation of certain mRNAs when a dendritic cell becomes activated.

I consider it likely that the mRNAs in question are not randomly distributed throughout a dendritic cell's cytoplasm. If this is so, these cells may contain a 'translational hotspot' of requisite proteins and enzymes around each pathogen-containing vesicle, required for the orderly handling of the newly ingested microbe. The result might be the creation of an intracellular solid-state 'device' specifically for the processing and presentation of that microbe's antigens.

Discuss this paper at **http://blogs.nature.com/nature/journalclub**

NEWS



The latest analysis from the US National Science Board confirms that Israel leads the world in its economic devotion to research and development (R&D). Its civilian R&D spending in 2005 accounted for 4.71% of gross domestic product (GDP), more than twice the average among members of the Organisation for Economic Co-operation and Development (OECD).

Although US R&D investment was the world's largest — $340 billion — and in 2004, it was more than that of the rest of the G7 nations combined, the report offers some evidence of a slight decline in its standing. Its 2.57% share of GDP is comfortably above the OECD average of 2.25%, but both South Korea and Switzerland have leap-frogged ahead of the United States by this measure since the board's previous report in 2006. Germany could now be poised to do the same.

Most countries are investing more in R&D than they were, says Arden Bement, director of the National Science Foundation, which published the report. For example, although China ranks 23rd in GDP share — just 1.34% — it has pulled ahead to third in total R&D investment with an estimated $115 billion in 2005. ■

Rachel Courtland

International genome project launched

A much-anticipated international project to sequence the entire genomes of 1,000 people was launched on Tuesday, but some question whether the three-year project is ambitious enough in its scope.

The '1,000 Genomes Project' will create a highly detailed reference map of human genetic variation and is the largest such project announced to date. "This is a historic turning point in genomics," says Yang Huanming, director of the Beijing Genomics Institute, whose Shenzen branch is one of the three institutes launching the project. The other two are the Wellcome Trust Sanger Institute in Cambridge, UK; and the National Human Genome Research Institute in Bethesda, Maryland.

The project is expected to cost just US$30 million to $50 million — a fraction of what it would cost if they used the 'older' technologies used in the Human Genome Project. Instead, the initiative will use 'next-generation' sequencing technologies, although these are still being tested. "Projects such as this drive technology development," says David Altshuler, a geneticist at Massachusetts General Hospital in Boston. The project leaders have not yet decided what the depth of coverage of the genome will be — that is, how many repeats they will carry out for each chromosome.

"Projects such as this drive technology development."

All the participants in the first phase of the project will be drawn from the International HapMap Project, a large study on genetic diversity, although more people may be recruited later. HapMap has guided scientists to hundreds of 'single nucleotide polymorphisms', or SNPs — places where people's genetic codes differ by a single DNA base — in genetic regions associated with disease. But these associations explain only a small part of an individual's risk for any particular disease. And scientists must undertake large, expensive follow-up studies to hunt down the specific causes of disease risk lurking in these genetic regions.

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The new project aims both to guide scientists towards more disease-associated regions, and to hasten much of the costly follow-up research. Sequencing 1,000 individuals will allow scientists to look at more types of variation — most notably, structural variation, in which large stretches of DNA are duplicated, deleted or rearranged in different individuals. And it will capture more rare variants than the HapMap, which aimed to catalogue SNPs present in 10% of the human population.

"This will give us a much more complete catalogue of genetic variation, and that is going to have a profound impact on our ability to understand the risk factors underlying disease," says Francis Collins, director of the National Human Genome Research Institute.

Yet some scientists question how accurate the finished genomes will be, given the project's short timeline and low budget. Others say that the project should have included some phenotypic information about the participants — such as medical records or basic data such as height and weight. "It's curious that the disease-association studies don't exploit much sequencing — and the sequencing studies don't use the disease data. It would be helpful to hear a clear explanation of why, after 17 years and billions of dollars, these studies still aren't coordinated," says George Church, who is leading a venture called the Personal Genome Project out of his lab at Harvard University in Cambridge, Massachusetts. Church's project is collecting and releasing genetic and phenotypic data on ten individuals, including himself.

"This is a historic turning point in genomics."

But leaders of the 1,000 Genomes Project say that their venture isn't large enough to give definitive answers about the genetic roots of traits. They also say that collecting phenotypic information might bias their study and raise difficult issues, such as how to protect participants' identities while still releasing all the relevant data. The project will not collect or release any information about its participants, beyond their ethnicity and genome sequence.

"No single study with 1,000 people is going to contain enough individuals with any condition to give you any power at all to say whether genotypes or phenotypes are correlated," Collins says. Such work is best left to follow-up studies, he says. ■

Erika Check Hayden

Q&A: Larry Brilliant

R. GALBRAITH/REUTERS

He's a physician who has had a major role in the eradication of smallpox and in tackling blindness. Now Larry Brilliant is heading up Google.org, the dotcom giant's philanthropic arm, which plans to tackle emerging diseases, climate change and poverty. **Declan Butler** talks to him about his diseases strategy.

How will the organization work to anticipate new pandemics?

With our 'Predict and Prevent' initiative we hope to develop an entire new science of epidemiology and surveillance, both for existing diseases and to spot emerging ones early on. One way is to strengthen national health services — look at the polio surveillance system in India, for example, which is the finest for any disease. We are now funding the Global Health and Security Initiative's work on the Mekong Basin Disease Surveillance network, to boost diagnostic capacities, train people and help create a regional surveillance network for this hotspot, which covers Cambodia, China, Laos, Myanmar, Thailand and Vietnam. It is also using tabletop planning exercises with modern war-gaming techniques to better prepare a pandemic response. The Mekong project is about creating best practices that can then be transposed elsewhere. It's all about sharing data, visualizing data and creating the IT tools that people would like these countries to have to mount fast and effective responses.

But governments aren't always open about reporting disease outbreaks.

That's why we are supporting projects that use information technology to sift through news reports, blogs, electronic hospital records and other sources, in multiple languages, to identify reports of disease before official government reports. And we are supporting another project that enables individuals and health workers to report cases directly using cell phones to create a global network — this is going to be a big movement. So we've given $5 million to InSTEDD [Innovative Support to Emergencies, Diseases and Disasters], and $450,000 to HealthMap to start developing such systems. It's a new and embryonic field, it's exciting, and it's an area that Google.org wants to have a role in. We would like to support all of the players in this space to nurture a new industry. We are just at the beginning here.

I see Google is also involved in biology.

We want to detect emerging pandemic agents. Humans are increasingly coming into closer contact with animals in many places, creating hotspots where new diseases emerge by jumping the species barrier. So we plan to support work that takes paired blood samples from animals eaten for bushmeat in Africa, and from their hunters. This will create genomic maps of the viruses present, and reveal how these agents change over time. It's part of an entirely new chain of information gathering, which at some point will need to be centralized by the World Health Organization, the World Organisation for Animal Health or a non-governmental organization.

... and Earth-monitoring data?

We're also supporting Clark Labs at Clark University, with equal funding from the Gordon and Betty Moore Foundation, to develop systems to see how long-term Earth-monitoring data sets on climate change and variability, and soil moisture can be used to predict changes in ecosystems in Africa and the Amazon, and disasters such as drought and famine. An El Niño event might, say, predict a later drought in Mozambique. I'm a recent convert to this field of teleconnections. We want to advance this field of science. It is not yet well understood.

How are your relations with the Bill & Melinda Gates Foundation?

I love the Gates Foundation, they are wonderful people, and we will work with them. They have been so kind to me and Google.org. They invited us round to help us understand philanthropy and how it can best be used. When I heard Bill and Melinda speaking recently on their commitment to malaria, I had tears in my eyes.

The grants Google.org announced last week were small compared with those made by the Gates Foundation's Global Health Program.

It's too early for substantial funding but Google has set aside a generous amount for philanthropy — 1% of equity and 1% of profit. We've invested a total of US$75 million so far, but I consider these mostly exploratory grants. It is the beginning of a long process and we will be ramping up giving in the future. ■

SPECIAL REPORT

Nuclear fuel: keeping it civil

In the third of a series of articles examining nuclear issues, **Jeff Tollefson** looks at options for fuelling a global boom in nuclear power stations without enabling nuclear proliferation.

Billionaire investor Warren Buffett put US$50 million on the table in 2006 and challenged the world to raise another $100 million for an international nuclear fuel bank. He called it "an investment in a safer world" — a world that will soon host an expanding group of nuclear power reactors. The idea was developed by the Nuclear Threat Initiative (NTI), a charity based in Washington DC that works to reduce the dangers of nuclear technology, and the proposal has met with enthusiasm from politicians, think-tanks and bureaucrats.

But the offer expires in September, and so far only the United States has stepped up, tucking $50 million into a $555-billion spending bill passed by Congress last month.

The premise behind an international fuel bank is that it will encourage countries who are building nuclear reactors to forgo uranium-enrichment technology because such technologies also provide most of the means for developing nuclear weapons.

Dozens of countries are starting to express an interest in nuclear energy. And established nuclear powers that had all but abandoned new plants are laying the groundwork for a new boom. Plans to expand enrichment are underway among the biggest nuclear-energy suppliers — the United States, Russia, France, the Netherlands, Germany and the United Kingdom.

"Any legislative attempts to impose limits on enrichment technology are going to fail", warns Laura Holgate, a vice president at the NTI. "The challenge is to create attractive alternatives to indigenous fuel cycles," she says. One such alternative is to make enriched fuel available without hindrance to any country that agrees not to make it itself.

Mohamed ElBaradei, director general of the International Atomic Energy Agency (IAEA) helped to kick-start the discussions for an international fuel bank in 2003. He proposed a system in which all uranium enrichment and fuel would be placed under international control. Since then, numerous countries and organizations have sent in proposals, all of which are still before the IAEA board of governors for debate.

Tariq Rauf, who heads verification and security-policy coordination at the IAEA, explains that the fuel bank would be a "reserve of last resort", where countries could purchase two or three years of fuel if they had their supply cut off as a result of some sort of diplomatic pressure. It would thus allow countries some assurance of continued access to fuel even if aspects of their non-nuclear policies were unpopular. Their supply would be severed, though, if there were a fear that they would use the fuel for proliferation purposes. "We wouldn't supply a country if we were investigating that country for safeguards issues," Rauf says.

A virtual bank

The NTI proposal would purchase enough fuel to fill one reactor core, which would fit into three semi tractor-trailers (lorries) and could be parked in any country. Alternatively, the fuel could be left in place with a guarantee for future IAEA access. Such a 'virtual' fuel bank might seem easy to set up but there are fears that it would be subject to the kind of political disputes that the fuel bank is supposed to avert.

"The fuel bank is a worthwhile thing to do, but its importance should not be exaggerated."

Unsurprisingly, most of the fuel-bank proposals emanate from the major nations that already supply the current commercial nuclear market; other countries have been less enthusiastic. South Africa and Brazil, for instance, question the very premise, suggesting that the proposal would widen the divide between nuclear and non-nuclear states by eroding the right to pursue enrichment for peaceful purposes, agreed under the Treaty on the Non-proliferation of Nuclear Weapons (NTP).

"The fuel bank is a worthwhile thing to do, but its importance should not be exaggerated," says Matthew Bunn, a non-proliferation expert at Harvard University in Cambridge, Massachusetts. Most of the countries that would tap into such a bank are likely to be upstanding members of the NTP and have access to the international market, he says. "Aside from India and Iran, most countries have very secure fuel supplies through the commercial market." And countries such as South Korea seem content to take their chances on the current market.

Meanwhile, Russia is moving forward with a proposal to create what it calls an international uranium-enrichment facility that would be placed under IAEA safeguards in Angarsk, near Lake Baikal in Siberia. Partners will be able to ship their own uranium to the facility and benefit from either the fuel itself or the sales. Russia has already signed an agreement with Kazakhstan and others are expected to join. It has also agreed to make a stockpile of roughly two reactor-core loads available to the IAEA, but experts are not clear what kind of non-proliferation requirements might be imposed on countries seeking to use its stockpile or to participate at Angarsk. The United States has proposed creating its own stockpile of about 6.5 reactor-core loads, but that fuel would be available only to countries that refrain from uranium enrichment.

Holgate says that these proposals complement the international fuel bank by addressing another possible motivation for developing enrichment technologies: the desire to make

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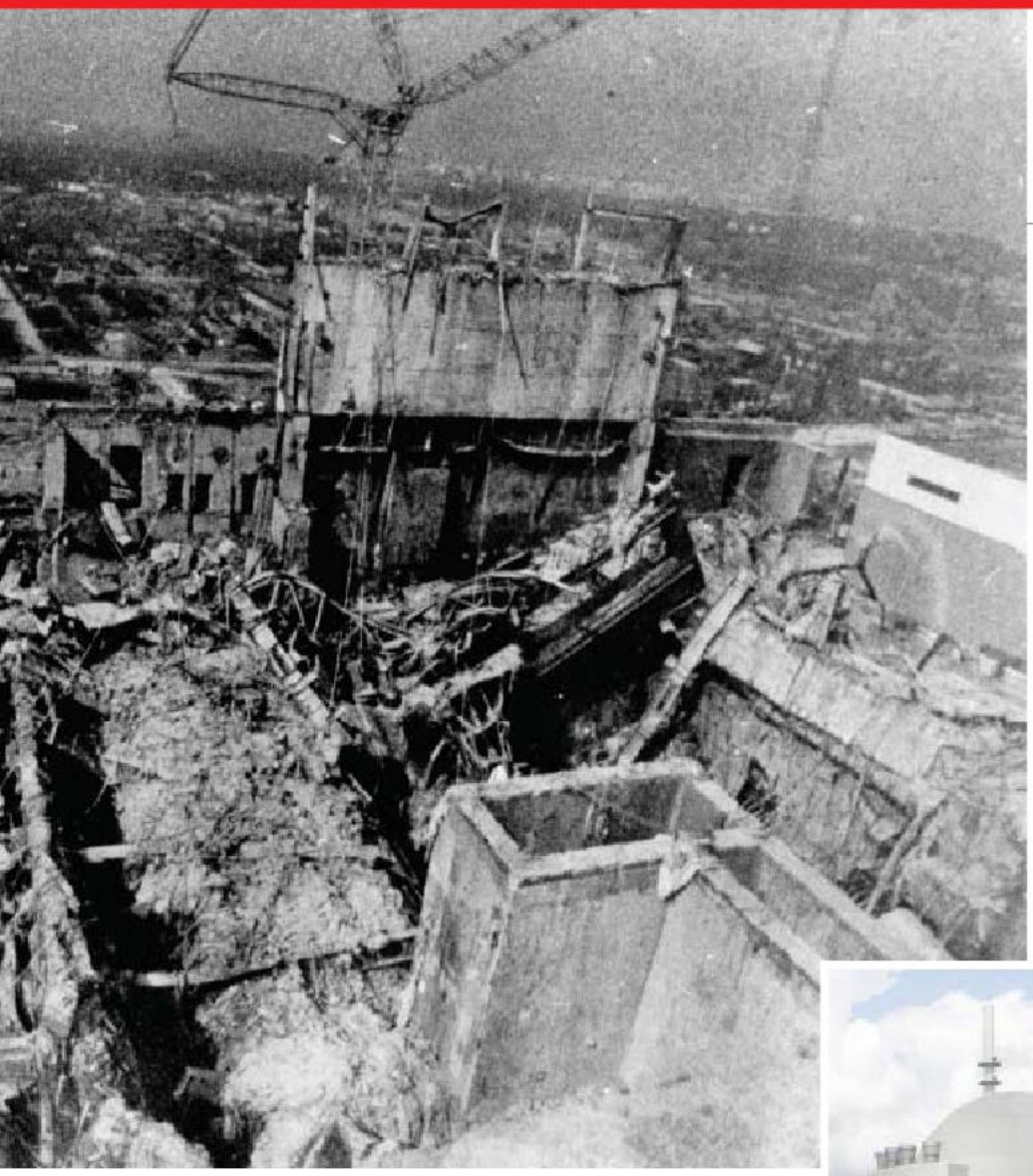
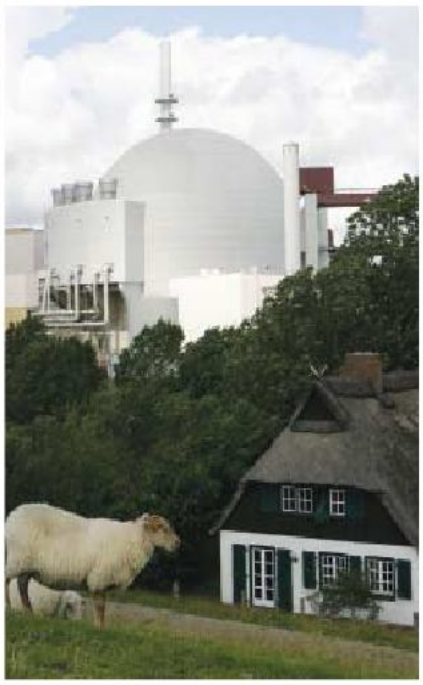
C. ROBINSON/BRITISH ANTARCTIC SURVEY



Nuclear power: another Chernobyl-style disaster in progress or the promise of a greener future?



I. KOSTIN/SYGMA/CORBIS

M. ROSE/GETTY

money in what is presently a closed international market. "If you are a wool producer, you would rather sell the cloth than the wool, or better yet sell the suit," she says.

Even if none of these proposals alters the equation on its own, together they might help to expose the hedgers by removing the most plausible reasons for uranium enrichment. From this perspective, Holgate credits Russia's recent decision to provide Iran with fuel to aid its nuclear-energy programme as "exposing concerns that Iran's enrichment is questionable at best".

No guarantees

In the end, there is nothing to prevent a country such as Iran from moving forward with enrichment because it doesn't want to depend on major nuclear powers for its energy — or because it wants to develop its nuclear-science base and keep its options open for a future weapons programme. "It's all in the eye of the beholder," says Bill Potter, director of the James Martin Center for Nonproliferation Studies at the Monterey Institute of International Studies in California. "The same technologies are applicable to nuclear weapons and power — the key issue is understanding countries' motives."

Perhaps the best way to promote a broad expansion of nuclear energy would be to not just guarantee the fuel but also take back spent fuel so that new nuclear nations don't have to worry about storage. Such a 'fuel-leasing' programme would provide a big incentive for nations to enjoy nuclear power, but the politics are tricky because nobody wants to deal with somebody else's trash, says Bunn.

The Bush administration unveiled an ambitious solution in 2005, envisioning an international alliance that would lease fuel to non-nuclear nations and then take it back for reprocessing. The plutonium could then be used in advanced reactors by legitimate nuclear-weapons states. But this 'Global Nuclear Energy Partnership' was roundly panned by scientists, including a panel convened by the US National Academy of Sciences, as being too risky, premature, expensive and overly ambitious. Last month Congress scaled it back to a research and development programme, although the Energy Department continues to push the idea internationally.

Law changes

President Bush has also reversed US policy on India, a pariah on nuclear issues since its first nuclear test in 1974; India declined to sign the NPT and has been blacklisted on the commercial market ever since. Although it has received aid from Russia in recent years, India has struggled to provide fuel for energy and for its weapons programmes due to a paucity of domestic sources. In 2006, Congress amended US law to allow nuclear trade with India as long as it does not contribute directly to the weapons programme. But the deal is now under assault by the Communist Party in India because of concerns about the loss of sovereignty and the level of US influence over the nation. The Indian government is nonetheless pushing forward in negotiations with the IAEA to bring more — but not all — of its nuclear facilities under IAEA safeguards. The Nuclear Suppliers Group, which represents major nuclear exporters, must also change its rules to allow exports to India, a move that would open the entire international market to India.

The country might need that access if it wants to fuel a new fleet of nuclear power plants in the coming decades. Western companies in the Nuclear Suppliers Group also stand to reap the benefits. From a non-proliferation standpoint, however, there is nothing to prevent India from purchasing fuel internationally and then diverting its domestic supply to the weapons programme. It is this kind of situation that makes Potter question whether the world is ready for an expansion of nuclear energy.

"Are we simply cheerleaders? Or are we open-minded critics looking at the positive and negative consequences of a significantly expanded global nuclear environment?" he asks. "I worry that commercial considerations will trump non-proliferation, nuclear-terrorism and nuclear-safety considerations." ■

See Editorial, page 373.

A very mysterious foundation

Some 3,000 scientists, including more than 100 Nobel laureates, have apparently accepted membership of a body called the World Innovation Foundation (WIF), which claims to be a powerful world-changing network to provide "the technological tools and miracle technologies that we shall all need to solve the world's impending global problems".

No fewer than four Nobel laureates hold executive positions in the WIF's governance, according to its website: Jerome Karle, William Knowles, Robert Huber, and Yuan Lee. Huber, described as vice-president, claims that he has no recollection of joining the organization. "I am not aware what this organization is," he says.

Metaphysical: the proposed US$22.5 billion Open Research Establishment. C. WADE

Yuan Lee, a 1986 Nobel laureate in chemistry, says he has had "very limited" involvement with the foundation that amounted to accepting a 2002 invitation to join, signing a WIF letter opposing the Iraq war, and accepting in 2004 the position of the WIF 'national representative' in Taiwan. Since accepting this position he has had no dealings with the WIF, he says.

Nature polled several WIF fellows who advertise their fellowships on their websites. What emerges is a pattern whereby scientists join on the strength of the list of existing members, but know little about the foundation or its activities. "I was invited under the signature of a Nobel laureate. It looked as if it was going to do some good in developing countries," says Satya N. Atluri, the Samueli/Von Karman aerospace engineering chair at the University of California, Irvine, who accepted a fellowship. "So far, I am involved in nothing."

> "There is no way of knowing whether the WIF is active or defunct."

The WIF, which has no full-time staff or offices, was founded by David Hill, who runs a construction consultancy in Huddersfield, in West Yorkshire, UK. He first registered the WIF as a series of private UK companies in the 1990s, and then in 2005, as a foundation in Bern, Switzerland. Hill, who holds an honorary doctorate from the National Academy of Kyrgyzstan, continues to operate the WIF from Huddersfield alongside his private business and says he spends half his time on the foundation free of charge.

Bern's Chamber of Commerce lists the WIF's current legal structure as a foundation with four directors: Hill; Lutz Baehr, former executive director of the African Development Bank; Christopher Leuch, a Bern-based attorney specializing in tax matters; and Dialog Treuhand, a Bern-based accounting firm. Although a charity, its accounts are not public. Under Swiss law a foundation has no obligation to publish its accounts, says Leuch. Hill says he was "not aware that the accounts were not available", but that he must wait on incoming board members to decide if he has permission to show them.

"Members of the future WIF board are aware of WIF's structural and financial shortcomings, but we hope to overcome these this year," adds Baehr. "To draw, however, from these existing shortcomings, conclusions that question the state of the WIF, will not do justice to this process, to the integrity of those involved in this process, or to the cause of the WIF and its steadily growing fellowship."

The promised land

Among the WIF's stated goals are the creation "for the future benefit of all humankind" of the ORE-STEM Complex, a proposed US$22.5 billion proposal it describes as a "scientific, technological, engineering and manufacturing complex — the world's largest open-research establishment. Equipped and operational for 20,000 leading-edge scientists, engineers, technologists and innovators". It has been a goal for a decade, but so far it exists only as a web page on the WIF's site. The plan is still in a preparatory phase, says Hill, "the drawings and documents for the ORE-STEM are confidential".

Brian Ralph, a materials scientist who is now

Creationists launch 'science' journal

The organization that last year opened a US$27-million creation museum in Kentucky has started its own 'peer-reviewed' scientific research journal.

On 9 January, Answers in Genesis, a Christian ministry run by evangelical Ken Ham, launched *Answers Research Journal* (*ARJ*), a free, online publication devoted to research on "recent Creation and the global Flood within a biblical framework". Papers will be peer reviewed by those who "support the positions taken by the journal", according to editor-in-chief Andrew Snelling, a geologist based in Brisbane, Australia.

"There have been these kinds of publications in the past," says Keith Miller, a geologist at Kansas State University in Manhattan, who follows creationism. For the most part, he says, the work is ignored by the scientific community. But those without a science background, including some policy-makers, may not be able to judge the difference in value of a paper in *ARJ* and a genuine science journal.

Recent court rulings make it all but impossible for intelligent design, a belief that a higher being shaped evolution, to be taught in US public schools. Nevertheless, creationists still try to discourage the teaching of evolution and other scientific theories at the local level, according to Eugenie Scott, executive director of the National Center for Science Education, an education watchdog in Oakland, California. Publications such as *ARJ* are part of the continued battle to excise science from local

retired from Brunel University in Uxbridge, UK, was among the first people invited in those early days and served on the WIF's board. But he became quickly disillusioned, he says, and has had nothing to do with the WIF since. "My impression was that Hill had lots of big ideas, but few results," says Ralph, adding that "Hill did seem to be able to attract people to go on headed notepaper by the bucketload".

The foundation's main current activity is providing scientific advice on sustainable development to governments worldwide, says Hill. He seems to be a one-man Davos conference, dropping names of ministers, ambassadors and heads of states that he has met, noting nonchalantly that he had a chat with Mikhail Gorbachev recently at a Nobel peace summit.

The foundation does have at least one project in the public domain. Baehr is testing a Vietnamese herbal remedy for heroin addiction — a project he began at the United Nations (see *Nature* 433, 568–569; 2005). The WIF has helped to open doors, says Ludger Wessjohann, an organic chemist at the Leibniz Institute of Plant Biochemistry in Weinberg, Germany, who is involved in the project. Hill says the WIF also has projects spanning from avian flu, to bringing solar electricity to rural Africa, to building research centres in Kazakhstan and Kyrgyzstan. But it is difficult to find out more, because the details, says Hill, are "confidential".

"It is a mysterious organization," says Ian Pearson, a WIF fellow and a former futurologist at British Telecom, "I was invited to join a couple of years ago and allowed them to re-use one of my articles for their web site, and that is the last contact I had ... It says at the outset that it works in secrecy, so of course there is no way of knowing whether it is extremely active or totally defunct." Pearson adds that he is likely to remove the WIF honours from his website. ■

Declan Butler

curricula, she says. "Creation science is alive and well and appealing to a substantial minority of the American public."

Miller, himself an evangelical Christian, says that scientists must be careful when responding to the launch of *ARJ*. Taking too strong a stand against the journal will fuel creationists' accusations of scientific 'bias' against religion, he argues. Researchers should instead try to educate non-scientists about the scientific process, he says. ■

Geoff Brumfiel

D. J. GREEN/ALAMY

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Huge crystal baffles chemists

A giant molecular bauble, by far the largest single-molecule crystal ever made, is confounding chemists. Involving almost 500 silver atoms, the crystals are so large and complex that their creators cannot figure out their structure.

Each of the 30 crystals made by Dieter Fenske of the University of Karlsruhe in Germany and his colleagues is thought to contain 490 silver atoms linked by 188 sulphur atoms and 114 organic groups, $Ag_{490}S_{188}(StC_5H_{11})_{114}$. "This is an idealized structure" based on energy calculations, explains Fenske, who published the structure this month (C. E. Anson *et al. Angew. Chem. Int. Edn* doi:10.1002/anie.200704249; 2008).

"With structures that size you are pushing the crystallography technique right to its limit," says chemist Paul Raithby at the University of Bath, UK. Yet, he adds: "As far as crystallography and mass spectrometry can prove anything, this structure is as definitively proved as you could get."

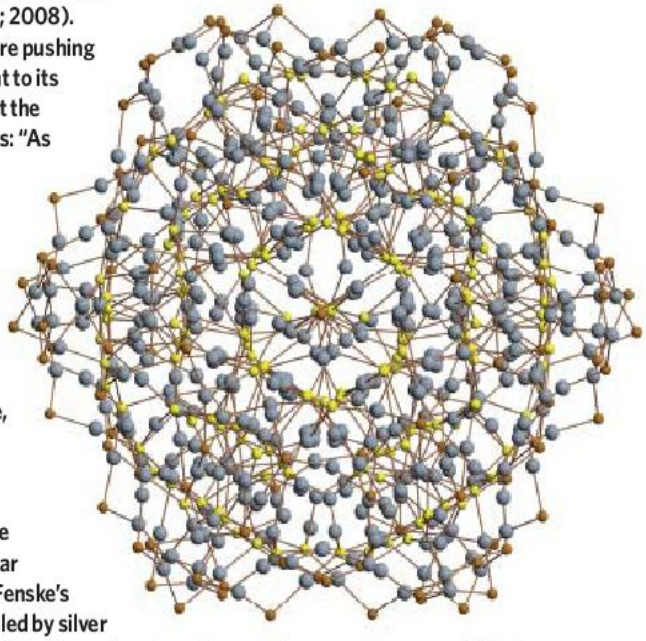
Fenske's molecules, described as 'clusters', produce crystals that are about 3 nanometres in diameter. The crystals have a well-defined outer 'shell' that is possible, though not simple, to characterize using X-ray diffraction.

But delve beneath the crystals' outer shell and things get a lot more complicated. Rather than the regular structure seen in a typical crystal, Fenske's clusters contain disorder: a void, filled by silver and sulphur atoms linked together in haphazard disarray. Molecules of silver sulphide (Ag_2S) can be arranged in a number of different geometries, including cubic, octahedral or dodecahedral. The disorder inside Fenske's cluster is so great that he can't tell what the geometry is in any of his samples. He describes the interior as looking "molten".

The crystals have been tricky to define in more than one way: even though they grow in a crystalline way, the disordered core causes a problem. "If the interior of the particles is 'mobile' then they are not crystals," says Frank Leusen, a crystallographer at Bradford University, UK. And are they molecules? Some say yes, others are not so sure. "These systems are at the boundary between molecular chemistry and bulk materials chemistry," says Raithby. "I am at a bit of a loss finding an appropriate term," adds Leusen.

Because of their huge size, Fenske's clusters may have characteristics that transcend the limits of molecular chemistry and enter the realm of macroscopic particles. For example, they may have interesting electrical properties.

The sheer size and complexity of the clusters mean that their internal structure cannot be revealed using X-ray diffraction. The technique uses X-rays that are reflected off the atoms in a rotating crystal, creating patterns of spots. These spots are converted into a map of the electron density around the atoms, and eventually the molecule's structure is calculated. But with larger molecules the number of spots increases, the spots get closer together and can even overlap. Also the intensity of each spot decreases as molecules get bigger.



Atomic mess: the exact structure of this giant crystal may prove impossible to determine.

Fenske instead calculated likely arrangements for the different internal geometries that Ag_2S could adopt within the known molecular mass. The most tightly packed arrangement, Fenske calculated, involves 490 silver atoms, making it by far the largest cluster reported.

"The research in this paper is spectacular," says Bill Clegg, a crystallographer at Newcastle University, UK.

But Fenske is not stopping there. In the same mixture that produced the black crystals he thinks he has a cluster that contains 800 silver atoms, the structure of which he is grappling with at the moment. "I'm pretty sure we can get larger particles," he says. ■

Katharine Sanderson

MRC Medical Research Council

RSA Executive Search

Director of the Mary Lyon Centre

Harwell

Since its inception in 2004, and led by Professor Bob Johnson, the Mary Lyon Centre (MLC) has developed state-of-the-art mouse genetic facilities within the MRC Harwell Campus, a leading international centre that includes the Mammalian Genetics Unit (MGU). The MLC and MGU are co-located with the new Synchrotron facility, DIAMOND, and other significant biomedical research facilities under development. With a current total annual budget of £6m, and a staff complement of circa 100, the MLC has successfully established a variety of services in mouse genetics and functional genomics underpinning Harwell's leading role internationally in the generation of mouse models of human disease.

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STANFORD SCHOOL OF MEDICINE
Stanford University Medical Center

Chair, Department of Genetics

Stanford University School of Medicine

The Stanford University School of Medicine is initiating a search for the position of Professor and Chair for the Department of Genetics.

The Stanford University School of Medicine is situated adjacent to the School of Humanities and Sciences, the School of Engineering, and two internationally-renowned adult and pediatric hospitals (Stanford Hospital and Clinics and Lucile Packard Children's Hospital), fostering a highly integrated approach to interdisciplinary science that is the hallmark of Stanford University. The School of Medicine is seeking an accomplished scientist in the fields of genetics, genomics, or both, with the creative vision to lead and shape the future of a distinguished and innovative Department of Genetics.

Stanford University is an equal opportunity employer and is committed to increasing the diversity of its faculty. It welcomes nominations of, and applications from, women and members of minority groups, as well as others who would bring additional dimensions to the university's research, teaching and clinical missions.

Interested candidates should submit a CV and letter of interest to the search committee by February 29, 2008 to:

Lucy Shapiro, PhD, Chair
Committee to Search for a Chair of the Department of Genetics
c/o Kendra Baldwin
Office of Institutional Planning
Stanford University School of Medicine
Building 110, Room 1
555 Middlefield Road
Menlo Park, CA 94025
or by email to:
kendra2@stanford.edu

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MINISTERIO DE EDUCACIÓN Y CIENCIA

THE BALEARIC ISLANDS COASTAL OBSERVING SYSTEM (SOCIB)

seeks a

DIRECTOR

The BALEARIC ISLANDS COASTAL OBSERVING SYSTEM (SOCIB) is a new research and technology facility for operational oceanography, created as a Consortium of the Spanish Ministry of Education and Science and the Balearic Islands Government.

The mission of SOCIB is to develop a state-of-the-art Observing and Forecasting Operational Oceanography System, a scientific and technological infrastructure which will be open to international access and collaboration.

The Director of SOCIB reports to the Governing Council and is responsible for managing the Coastal Observatory implementation and operations, and for maximizing its readiness and effectiveness for scientific research and technology development. The Director is responsible for recruiting and maintaining high quality scientific, technical and administrative staff, developing an annual budget for review and approval, and proposing the short and long-range plans and priorities for SOCIB.

Salary range and starting date are to be negotiated.

Review of applications will begin in February 2008, and the recruitment will remain open until the position is filled.

Additional information of characteristics and conditions of the position on **http://socib.balearsfaciencia.org/**

W121801R

Cellular memory hints at the origins of intelligence

Learning and memory — abilities associated with a brain or, at the very least, neuronal activity — have been observed in protoplasmic slime, a unicellular organism with multiple nuclei.

When the amoeba *Physarum polycephalum* is subjected to a series of shocks at regular intervals, it learns the pattern and changes its behaviour in anticipation of the next one to come[1], according to a team of researchers in Japan. Remarkably, this memory stays in the slime mould for hours, even when the shocks themselves stop. A single renewed shock after a 'silent' period will leave the mould expecting another to follow in the rhythm it learned previously. Toshiyuki Nakagaki of Hokkaido University in Sapporo and his colleagues say that their findings "hint at the cellular origins of primitive intelligence".

It is well-established that cells receive, interpret and adjust to environmental fluctuations, says microbiologist James Shapiro of the University of Chicago, Illinois. But if the results stand up, he says, "this paper would add a cellular memory to those capabilities".

The organism chosen by the Japanese team could scarcely seem less promising as a quick learner. *Physarum polycephalum* is a slime mould belonging to the Amoebozoa phylum. It moves at a steady rate of about one centimetre per hour at room temperature, but this changes with the humidity of its environment. It slows down in drier air, and Nakagaki's team used this sensitivity to stimulate learning.

The team found that when the mould experienced three episodes of dry air in regular succession an hour apart, it apparently came to expect more: it slowed down when a fourth pulse of dry air was due, even if none was actually applied. Sometimes this anticipatory slowdown would be repeated another hour later, and even a third. The same behaviour was seen when the pulses were experienced at other regular time intervals — say, every half hour or every 1.5 hours.

If the dry episodes did not recur after the first three, the amoeba's sense of expectation gradually faded away. But then applying a single dry pulse about six hours later commonly led to another anticipatory slowing in step with the earlier rhythm.

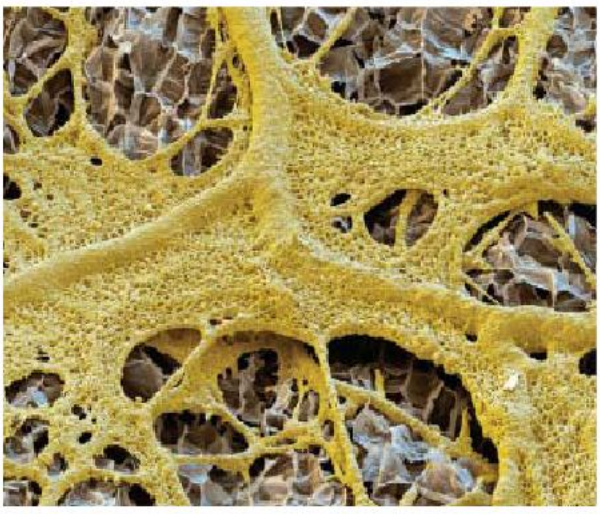
"The new finding adds to the cool things *Physarum* can do."

The same team has previously shown that these amoebae can negotiate mazes and solve simple puzzles[2,3]. So the new finding adds to "the cool things *Physarum* can do", says applied mathematician Steven Strogatz of Cornell University in Ithaca, New York.

Like all living organisms, slime moulds have built-in biochemical oscillators, like the human body clock. In other kinds of slime mould, these oscillators can create periodic ripple patterns in response to environmental stress, helping the organism coordinate its movements. Nakagaki's group thinks that the versatile rhythmic sense of *Physarum* stems from many different biochemical oscillators in the colony operating at a continuous range of frequencies.

The team's calculations show that such a group of oscillators can pick up and 'learn' any imposed rhythmic beat, although the knowledge decays quickly once stimulus ceases. The calculations also show that a memory of the beat can stay within the system, and be released again by a single, later pulse — just as the researchers observed. ■

Philip Ball



EYE OF SCIENCE/SPL

Slime moulds demonstrate primitive learning and memory.

1. Saigusa, T., Tero, A., Nakagaki, T. & Kuramoto, Y. *Phys. Rev. Lett.* **100,** 018101 (2008).
2. Nakagaki, T., Yamada, H. & Tóth, Á. *Nature* **407,** 470 (2000).
3. Nakagaki, T., Kobayashi, R., Nishiura, Y. & Ueda, T. *Proc. R. Soc. B* **271,** 2305–2310 (2004).

ON THE RECORD

"They behave in an entirely irresponsible and repellent way, which no doubt they did 50,000 years ago."

Eminent biologist Steve Jones doesn't like the people who populate his local London area of an evening, comparing them to prehistoric humans.

NUMBER CRUNCH

6,000 is the number of barks analysed by Hungarian researchers in an attempt to understand dogs' communication.

6 is the number of possible bark meanings identified by the researchers: stranger, fight, walk, alone, ball and play.

43% is the proportion of barks correctly translated by the researchers' software.

P. PONGRACZ

ZOO NEWS

Pricey python

What is thought to be the world's longest captive snake has been bought by a zoo in Ohio. Columbus Zoo and Aquarium paid US$4,800 per metre for the 7.3-metre python called, bizarrely, 'Fluffy'.

SCORECARD

▲ **Fear of flying**

Metal buried under the runway at London City Airport is causing "significant navigation problems" for aircraft taking off. One plane had to return to the ground after its instruments were disrupted.

▼ **Fear of clowns**

Some children's wards are reverting to having plain walls after researchers found that most kids find pictures of clowns scary, rather than amusing.

Sources: Camden New Journal, Telegraph, The Columbus Dispatch, Reuters, BBC

SIDELINES

NIH in the dark over conflicts of interest

The US National Institutes of Health (NIH) relies on an honour system that leaves it unaware of the details of situations in which its external grantees have financial conflicts of interest, according to a report released on 17 January.

The report, from the inspector-general of the Department of Health and Human Services, found that nearly half of the NIH's 24 grant-making institutes and centres were unable to provide any of the financial disclosure reports they received from external institutions between 2004 and 2006. Of the 438 reports that were produced, 89% were devoid of details describing the conflicts or how they were being managed.

Such details are not required under current rules; the report recommended that this change. But the NIH disputed that advice, saying that if it agreed to accept detailed reports, it would be held accountable for oversight duties that are properly the job of grantees' institutions.

T. DELFATTI/AUSTRALIAN ANTARCTIC DIVISION

A plane sits on the new ice runway in Antarctica.

Ice runway knocks weeks off journey to Antarctica

The first scheduled air link between Antarctica and Australia opened this month, giving Australian researchers a 4.5-hour trip to the frozen continent. Until now, they have had to travel for weeks by sea.

The first passenger flight carrying scientists landed on 11 January on a blue ice runway some 60 kilometres from the Casey research station. The initial trip made by air included researchers studying penguin ecology, and more than 20 scientists are expected to travel to Antarctica using the new flight path by the end of January.

The air link, which is operational between October and February, will run at least 10 flights a year. The Airbus A319 is fitted with long-range fuel tanks to allow it to travel to and fro without refuelling in Antarctica.

Government abandons bid to save US jaguars

S. WIDSTRAND/NATUREPL.COM

The US government will not attempt to save jaguars from extinction within the formal system of the Endangered Species Act.

The jaguar (*Panthera onca*, pictured) once ranged across the southern United States, but later essentially vanished, with the exception of a few males that have been seen slinking through New Mexico and Arizona during the past few decades. These outliers, according to a decision released on 17 January by the US Fish and Wildlife Service, do not justify a formal "recovery plan". The agency says that it will instead work on behalf of the endangered cat with other countries south of the border that comprise the rest of the animal's range.

"What is really important is to focus on the jaguars where they are," says Elizabeth Slown, spokeswoman for the agency's southwest office in Albuquerque, New Mexico. "I don't want people to think we are abandoning the jaguar." Environmental groups say the government is doing just that. Kieran Suckling, policy director of the Arizona-based Center for Biological Diversity in Tucson, called the move "a death sentence".

Early-stage human embryos cloned from adult cells

A Californian company says it has brought human cloning research to a new level with the efficient production of five cloned early-stage human embryos called blastocysts from adult skin cells.

Stemagen, which is based in La Jolla, hopes that its achievement will lead to the use of cloning techniques for biomedical research and, potentially, therapy. But first it will need to go the next step — using cells from a patient to generate blastocysts and then establish self-propagating lines of embryonic stem cells that, as clones, would be genetically identical to the patient.

Cloned human blastocysts have been reported before, but previously they have been made from human embryonic stem cells. The findings were reported last week in *Stem Cells* (A. French *et al. Stem Cells* doi:10.1634/stemcells.2007-0252; 2008).

UK government under pressure over physics cuts

The UK government is being forced to defend its funding levels for physics. Research grants are expected to be cut by at least 25% despite a budget increase of 13.6% over three years for the Science and Technology Facilities Council (STFC), which awards physics funding.

Documents obtained through the freedom of information act by a group of concerned physicists show that the STFC warned the government in advance that such cuts would mean fewer grants and reduced operation of key facilities.

Giving evidence to an inquiry on 21 January, Ian Diamond, head of Research Councils UK, the umbrella group for the country's research-funding councils, said the STFC would not be the only council affected by the budget changes. "I suspect there will be reductions in success rates [for research applications] across the board," he said.

European registry makes stem-cell use transparent

Europe has launched a registry to give researchers, regulators and the general public access to broad information about all available human embryonic stem-cell lines developed in Europe and their use.

Sponsored by the European Commission, the European Human Embryonic Stem Cell Registry went live on 18 January at www.hescreg.eu.

The repository will provide an efficient research tool. But the commission also hopes that it will make use of the ethically sensitive cell lines fully transparent in a region where each nation has different regulations. For example, scientists may derive new stem-cell lines from embryos if they are based in Britain, but not if they are in Germany, where the registry will be hosted.

Scientists involved in the project say that sharing information and materials may help limit the number of new lines generated.

Shattered illusions

Last-minute cuts to the research budget have left US scientists nervous about future funding. **David Goldston** looks at what Congress and the president might do next.

PARTY OF ONE

Trying to follow the actions of the US Congress on research spending in 2007 was like sitting blindfolded on a roller-coaster, and when the year ended in sudden free-fall, science lobbyists were shaken and stunned, and left wondering how they were taken for a ride. The year's victories — a supplemental spending bill in the spring that unexpectedly included additional science funding; legislation in the summer pledging to double research spending over 10 years; and spending bills in the autumn with long-sought increases for the physical sciences — proved to be no harbingers of a bounty for researchers when Congress finally completed its work in December. The final budget for research as a whole for fiscal year 2008 does not keep pace with inflation (see *Nature* **451**, 2–3; 2008).

What happened and what does it portend for the fiscal year 2009 budget process, which will begin when the president unveils his proposed spending plan on 4 February? First, it's important to recognize what did not happen: there was no sudden reversal of pro-science sentiment. Rather, the large increases initially destined for research agencies fell victim to a dispute between the president and Congress over the total amount of domestic spending (see *Nature* **449**, 962; 2007).

Once the president and his allies in Congress refused to budge on the size of the total spending pie, the Democrats in Congress had little choice but to redistribute some of the money slated for science to protect the full range of their priorities, many of which seemed more urgent or politically salient. Some of the money that had been allotted to the National Science Foundation in earlier bills, for example, went to the Federal Bureau of Investigation, which is in the same budget bill. Some of the funds for the Department of Energy's Office of Science went to the more applied-research programmes in the agency's Energy Efficiency and Renewable Energy division.

The theory one hears most often in Washington these days is that the budget increases for science were pared because the Democrats were unwilling to support any additional funds that had been proposed by the president. The Democrats' attitude towards the president was, supposedly: "You're unwilling to negotiate with us, so we're not going to provide money for any of your priorities." But congressional staff plausibly counter that the reductions resulted less from politics than from simple arithmetic — once the total pie shrank, even some favoured pieces had to get smaller. And in the final budget, most science agencies still received increases, whereas domestic spending as a whole was essentially frozen at last year's level — although that is cold comfort for scientists who had visions of sugar plums dancing in their heads.

What's next? Ironically, the fall-out from this year's final science numbers may put science in a stronger political position for fiscal year 2009. The reaction has been swift, vocal and angry, most importantly from high-tech executives, who have been assiduously courted by Speaker of the House Nancy Pelosi (Democrat, California). Stung by criticism from Silicon Valley immediately after the final spending bill passed, Pelosi's office took the highly unusual step of e-mailing a note to high-tech lobbyists, saying: "I want to assure you that my commitment to [science] remains strong and steadfast … [w]e didn't get all the research funding we hoped for this year. But rest assured we will be back fighting next year and for years to come to ensure" that funding is doubled over 10 years.

So far, the efforts at reassurance seem to have done little good. On the same day as Pelosi's missive, Craig Barrett, the chairman of Intel Corporation, sent her a short, blistering note that was not officially released but continues to circulate widely in Washington. He concluded: "Industry is listening carefully to your deliberations — if there is no government support [for the physical-science research and education] that will dictate our competitiveness for the next century then we might as well just accept that and make our investments elsewhere. I hope that Congress and the administration can find the will to make the right choices about where to spend our precious tax payers' monies." Barrett has been a leader in pushing for spending increases, but many high-tech executives share his concerns and are more willing to lay the blame for the cuts solely at the Democrats' doorstep.

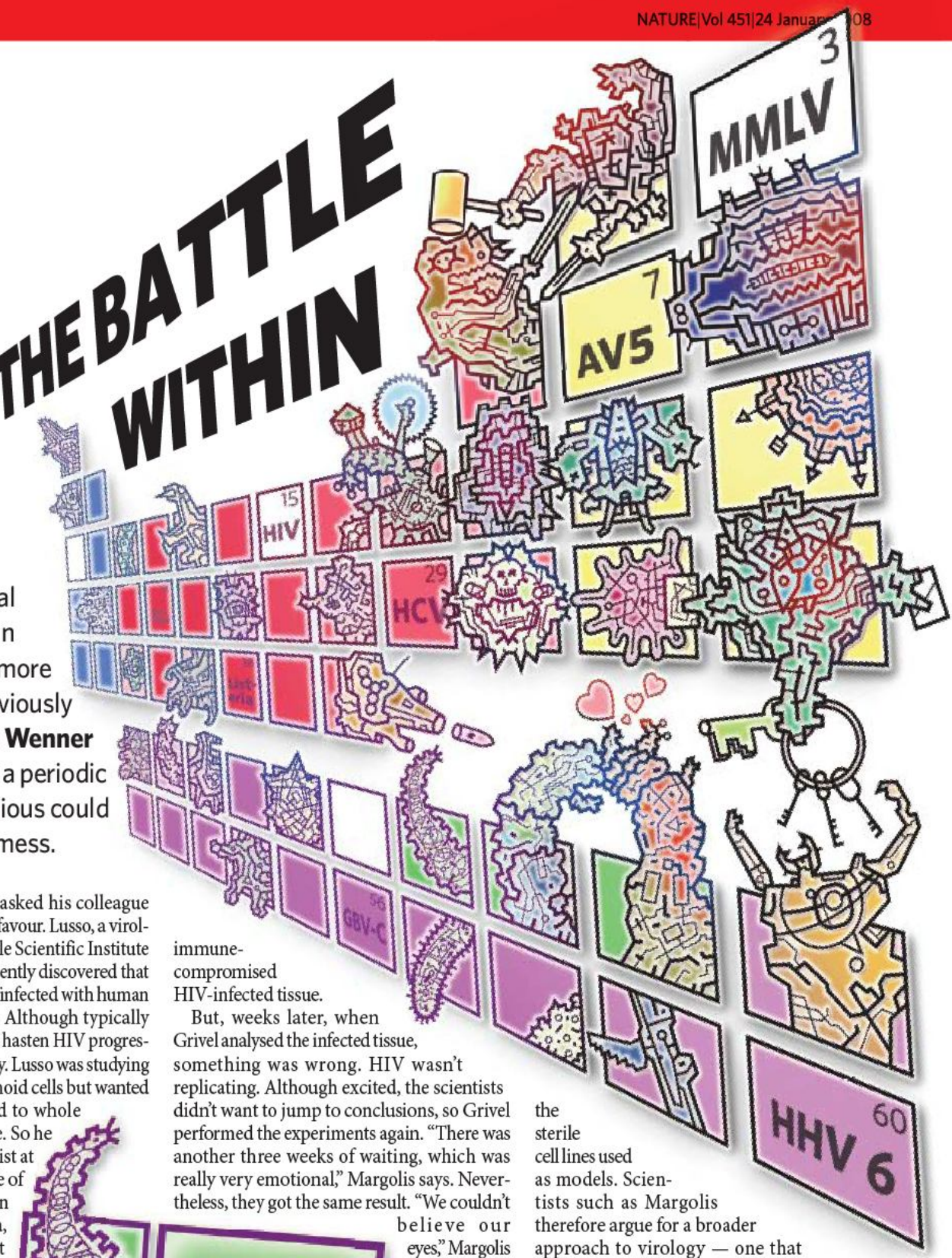
Especially in an election year, Democrats will be wary about alienating a key industrial constituency. At the very least, Pelosi's office is likely to keep a sharper eye on science spending than it seemed to in December's last-minute, frenzied budget negotiations. And science lobbyists are trying to figure out ways to capitalize on the Democrats' political concerns immediately by pushing to include science money in any supplemental spending bills that could yet add funding in the current fiscal year. That would be an uphill battle, as such bills usually focus on military spending or natural-disaster response. If any money were provided, national laboratories that are facing lay-offs, such as Fermilab in Illinois and the Stanford Linear Accelerator Center in California, would be the most likely beneficiaries.

But it's much too early to predict what will happen. Indeed, administration officials are still arguing over the president's proposal for fiscal year 2009. At issue is whether the president will propose increases for science based on what he had requested for this year, or whether he will ask for less, using the final congressional numbers as a base. And it seems a safe bet that he will again draw a line in the sand on total domestic spending, meaning that next year's budget deliberations could very well still be ongoing on election day in November.

With so much uncertainty, lobbyists and politicians on both sides of the aisle ought to be careful not to create another crisis of rising expectations about science spending. Fanning excessive hope only demoralizes the scientific community. This year's numbers were genuinely disappointing, but the sense of abandonment and betrayal that they have left in their wake is probably much more damaging than what could turn out to be a one-year delay in getting on a better spending trajectory. ■

David Goldston is a visiting lecturer at Princeton University's Woodrow Wilson School of Public and International Affairs. Reach him at partyofonecolumn@gmail.com.

THE BATTLE WITHIN



J. DEPCZYK

Viral and microbial interactions within living tissues are more complex than previously thought. **Melinda Wenner** explores whether a periodic table of the infectious could help sort out the mess.

In 2001, Paulo Lusso asked his colleague Leonid Margolis for a favour. Lusso, a virologist at the San Raffaele Scientific Institute in Milan, Italy, had recently discovered that HIV patients are often co-infected with human herpesvirus 6 (HHV-6). Although typically benign, HHV-6 seemed to hasten HIV progression, and no one knew why. Lusso was studying HHV-6's effects on lymphoid cells but wanted to see what the virus did to whole pieces of lymphoid tissue. So he asked Margolis, a virologist at the US National Institute of Child Health and Human Development in Bethesda, Maryland, and an expert on three-dimensional-tissue, to perform some experiments for him.

Margolis agreed. Human lymph-node tissue was hard to come by, but tonsils, which doctors remove from patients all the time, are also lymphoid tissue — and Margolis had developed an experimental tonsil-tissue system to study HIV pathogenesis. Because HHV-6 infection was often found alongside HIV, Margolis and his colleague Jean-Charles Grivel co-infected tonsil tissue with both viruses. He predicted that the herpesvirus, normally suppressed by the immune system, would be free to replicate in immune-compromised HIV-infected tissue.

But, weeks later, when Grivel analysed the infected tissue, something was wrong. HIV wasn't replicating. Although excited, the scientists didn't want to jump to conclusions, so Grivel performed the experiments again. "There was another three weeks of waiting, which was really very emotional," Margolis says. Nevertheless, they got the same result. "We couldn't believe our eyes," Margolis says. HHV-6, at least in this situation, seemed to protect against HIV[1].

> "If HIV patients had GBV-C, they were three times more likely to be alive at follow-up."
> — Jack Stapleton

So infectious agents interact with each other and with hosts in unpredictable ways. An average human body is rife with viruses, and benign and not-so-benign bacteria as well as 'endogenous retroviruses', which buried themselves in the human genome eons ago. This crowded house is a different beast from the sterile cell lines used as models. Scientists such as Margolis therefore argue for a broader approach to virology — one that involves studying infections in a more true-to-life context, predicting their interactions and sometimes taking the unexpected good with the usual bad that infections bring.

That microbes can benefit their hosts is by no means new. For example, bacteria living in the human gut are known to influence immune function, and help our body absorb nutrients. But only recently have scientists suggested that infectious viruses could provide their hosts with benefits as well. Viruses influence the host immune system in significant — and occasionally beneficial — ways, a concept that isn't surprising when one considers that they have been interacting with immune systems for millions of years.

Recently, Herbert 'Skip' Virgin, an immunologist at Washington University School of Medicine in St Louis, Missouri, infected mice with dormant viruses genetically similar to human Epstein–Barr virus and human cytomegalovirus. These viruses, he found, protected the mice from the bacterial pathogens *Listeria monocytogenes* and *Yersinia pestis*. Virgin and his colleagues suggest that the viruses upregulate the production of immune factors that prevent further infection rather than interacting directly with the microbes[2].

Helping or hindering?

The endogenous retroviruses that cemented their place in human history by infecting the eggs and sperm of our ancestors account for more than 8% of our genome, and some report that as much as half of our genome is composed of fragmented viruses. These viruses seem to influence immune function; for example, the susceptibility of mice to Friend virus, a strain of murine leukaemia virus, is controlled by two genes derived from endogenous retroviruses[3]. Some have proposed that endogenous retroviruses, long fixed in mammalian genomes, provide the immunosuppression that allows a fetus to develop in its mother's body, despite the differences between the immune systems.

Viruses also interact with each other directly, as Margolis discovered for himself in 2001. Similar viruses sometimes compete with each other, causing one to eventually 'win over' a cell and literally block infection by others. Viruses that are different enough from each other can co-infect cells cooperatively; in a process known as complementation, one virus provides another with a useful protein that it co-opts for its own use. Occasionally, viruses become dependent on other viruses. One such example is hepatitis D, which requires the presence of hepatitis B in order to replicate.

Margolis uncovered why HHV-6 prevents HIV replication under certain conditions. A subtype of HIV, most often found in early infection, generally gains entry into the cell by binding the receptor CCR5. When HHV-6 infects first, however, it instigates production of an immune chemical that binds to CCR5 receptors, blocking HIV's access. HIV can develop resistance to this chemical over time and HHV-6 co-infection may exert selective pressure on HIV to become immune-resistant, or switch to a different co-receptor — a change accompanied by increased HIV virulence. This explains the often poor prognosis of patients infected by both viruses.

Other human viruses influence HIV replication. Margolis found that human herpesvirus 7 (HHV-7) inhibits HIV replication, albeit via a different mechanism from HHV-6 (ref. 4). In 1998, two groups independently reported that HIV patients infected with a seemingly innocuous hepatitis-like virus called GBV-C live longer, although neither group knew why. The studies piqued the interest of Jack Stapleton, director of the University of Iowa's Helen C. Levitt Center for Viral Pathogenesis and Disease in Iowa City, who was, at the time, running an AIDS clinic while studying hepatitis C–HIV interaction.

"We thought it was a statistical fluke and that it wouldn't hold up in a larger study," Stapleton recalls. With access to hundreds of samples, Stapleton decided to replicate the GBV-C study on a group of 362 HIV patients from his clinic. What he found surprised him and confirmed the findings: "If patients had [GBV-C], they were three times more likely to be alive at follow-up," he says[5]. Stapleton's subsequent research, along with work completed by a group in Germany, has shown that a GBV-C peptide interferes with early replication of HIV, and that GBV-C, like HHV-6, increases production of an immune chemical that blocks HIV's entry into the cell.

"I fantasize about creating a periodic table of microbes."
— Leonid Margolis

Always dangerous

No one would suggest purposefully infecting individuals with a persistent infection to ward off HIV. After all, one of the hallmarks of viruses is that they evolve quickly. "Any virus that is not causing disease has the potential to cause disease," says Robert Gallo, director of the Institute of Human Virology at the University of Maryland School of Medicine in Baltimore, and the co-discoverer of HIV. That said, Gallo, who worked with Lusso on the HHV-6 discovery, and his team recently tested the immune chemical produced during HHV-6 infection as a vaginal microbicide in macaque monkeys and found that it significantly lowered risk of contracting a monkey-infecting version of HIV[6].

"Any virus that is not causing disease has the potential to cause disease."
— Robert Gallo

To make further progress, scientists need to expand which viruses they study, and how they study them, Margolis says. They need to use tissue systems that preserve immune function and cellular communication, because cells within tissues communicate with one another differently and have a different architecture than do cells that are cultured *in vitro*.

Moreover, Margolis suggests, scientists should study infections in tissues harbouring the same persistent viruses present in humans. "If you study a pathogen such as HIV in a cell-culture model where there is no immune system, there is no effect from other microbes that are in the normal state in an infected human," says Stapleton. "You're really missing important factors that will influence the pathogenesis."

What would be ideal, says Margolis, is a table, not unlike Mendeleev's table of the elements, for infectious agents. "I fantasize about creating a periodic table of microbes," he says. Each cell in the table might feature the name of the microbe, the immune factors it affects, the receptors it uses and the signalling systems it incorporates, he says. Just as the elemental version predicts how two substances react with one another, the periodic table of microbes would predict how two microbes interact in the human body.

"It's off the wall, but it could generate enormous insights," says Michael Lederman, the director of the Center for AIDS Research at Case Western Reserve University in Cleveland, Ohio. Other scientists say that although such a table may have some practical value, the concept is potentially more interesting as a catalyst for scientific ideas and approaches. Philip Murphy, chief of the Laboratory of Molecular Immunology at the US National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, says that scientists can only get so far with existing tissue and animal models. "Pathogens are very host-limited, so there is a whole range of human pathogens that you could never do an experiment with in mice models," he says. For example, although Margolis's lymphoid-tissue model is incredibly useful, he says, there are a number of pathogens that will never infect lymphoid tissue. Scientists will, in other words, need to get creative.

Expanding the study of virology in these ways is challenging for other reasons, too. It is difficult, for example, for a scientist with expertise in DNA viruses such as herpesviruses to study their interactions with RNA viruses such as HIV. But collaboration can help, and although broadening the context of virology might make experiments more complicated, it will also make them more realistic, Margolis says. "In a more complicated system, you probably can understand less," he admits. "But what you understand is really relevant." ■

Melinda Wenner is a science writer based in New York City.

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NATURE|Vol 451|24 January 2008

Still in the lead?

Half a century after its creation, the Pentagon's Defense Advanced Research Projects Agency is considered a paragon of government innovation. But some question whether it is still relevant. **Sharon Weinberger** reports.

Last year's DARPATech conference could not have been held at a more fitting place than a Disneyland hotel in California. Run by the Pentagon's research arm — the Defense Advanced Research Projects Agency (DARPA) — the August meeting was meant to tout the agency's unparalleled record of far-out technological innovation. Next to the theme park, which features Buzz Lightyear Astro Blasters and elaborate virtual-reality displays, DARPA's message — military technology meets science fiction — took on a positively surreal form.

On display were a robotic dog that can traverse rugged terrain and advanced human prosthetics that could someday be controlled by the brain. One presenter talked of putting micromechanical systems into larvae to create cyborg insects, and the agency's director, Anthony Tether (pictured, right, with flag), touted its upcoming robot car race, a competition that promised US$2 million to the team whose vehicle could drive itself fastest through a 97-kilometre 'urban challenge' course. Meanwhile, meeting organizers passed out chocolates emblazoned with DARPA slogans and jostled past the pink-clad army of Cookie Lee jewellery saleswomen attending their own conference elsewhere in the hotel.

The event was in stark contrast to the buttoned-down DARPA of the early years. Since its creation in 1958, the agency has been a major player in science and technology challenges facing US national security. During the cold war, its experts worked on everything from the space race to the Vietnam conflict. Most famously, it created Arpanet, the precursor to the Internet. Today, in an era when federal agencies talk about becoming more like the private sector, DARPA is uniquely regarded as a model of success. "It is the jewel in the defence-department crown," says former defence secretary William Perry, who served as the Pentagon's director of defence research and engineering — a position that oversees DARPA — in the 1970s.

But now, as DARPA turns 50, observers are asking whether the agency is still as relevant as it once was. The question is particularly pressing as other parts of the US government look to launch their own DARPA spin-offs, in fields from intelligence to homeland security to energy (see 'DARPA: the next generation', overleaf).

A lean, mean machine

With just 250 employees, most of whom serve a term of 4 to 6 years, DARPA is everything the federal bureaucracy is not: lean, innovative and dynamic. Its approximately $3-billion budget is directed at high-risk projects designed to provide the Pentagon with revolutionary advances. Unlike the military services, DARPA projects don't have to be tied to a specific need, and unlike grant agencies such as the National Science Foundation, it can fund risky ideas without going through peer review. That high-risk approach can lead to high payoffs: the agency counts satellite navigation, unmanned aerial vehicles and radar-evading aircraft among its major accomplishments. But it's also had some flops: laser weapons are still elusive, and a decade-long strategic computing initiative never reached its goal of artificial intelligence.

Even in its early days, DARPA struggled for purpose and legitimacy. President Dwight D. Eisenhower created the agency in response to the Soviet Union's launch of Sputnik. The aim was to jump-start space programmes by bypassing the traditional rivalry among the military services that had been developing competing and overlapping satellite technologies. But within a year, the Advanced Research Projects Agency ('Defense' was added to its name in 1972) lost its major mission when Eisenhower created the civilian space agency NASA. The defence department eventually transferred the remaining military space programmes back to the military services.

G. BLEVINS/REUTERS

But DARPA soon found its niche in other defence work. Its Project Vela, which began in 1959 and continued through the 1960s, developed seismic sensors and satellites with which to detect foreign nuclear detonations — proving crucial in monitoring China's early nuclear tests and eventually enabling the United States to negotiate a weapons-testing treaty with the Soviet Union. And Defender, its 1960s anti-ballistic missile defence research programme, led the Pentagon to reject an army-built system in favour of DARPA-supported technology.

The Vietnam war reinforced the agency's role as the place senior leaders would go looking for solutions. As US military involvement in the war escalated throughout the

NORTHROP GRUMMAN

Spirit in the sky: the B-2 bomber came out of stealth research in the 1970s.

Anthony Tether waves the chequered flag at Grand Challenge 2005 — DARPA's competition to develop fully automated vehicles.

1960s, DARPA became a focal point for counterinsurgency work through its Project Agile. The project incorporated an array of research efforts and experimental technologies from the serious — the now-infamous Agent Orange, a defoliant widely used by the US military — to the retrospectively silly — a jet belt designed to propel individual soldiers on the battlefield.

Jack Ruina, DARPA's director from 1961 to 1963, says that he never liked the Agile projects, calling them "gimmickry and gadgetry". But Charles Herzfeld, who served as director from 1965 to 1967, says that he embraced the agency's mission in Vietnam. DARPA's 'systems approach' to counterinsurgency has included finding ways to spot tunnels in Vietnam, tracing relationships between families in the Middle East and developing radar to patrol Iran's borders.

Broad scope

At the same time, the agency continued its forays into areas such as computer and materials science. It fostered relationships with academia by funding long-term university research and by its sponsorship of the JASONs, a group of mostly university scientists that provides advice on national security. After a dispute over who got to choose JASON members, DARPA dropped the JASONs contract in 2002, and the group's sponsorship shifted to the director of defence research and engineering (see *Nature* **416**, 353; 2002).

Historically, DARPA directors also facilitated the agency's cooperation with universities and industry; they have all been engineers or physicists, many with close links to academia. As the director typically has sole discretion to fund or cancel a particular programme, the head of DARPA has great power to shape the agency's direction. Tether, an electrical engineer with a doctorate from Stanford University in California, spent much of his career in defence jobs in the private sector, working at companies such as Science Applications International Corporation in San Diego, California, one of the top Pentagon contractors.

> "The best DARPA programme managers must have inside them the desire to be a science-fiction writer."
> — Anthony Tether

Tether says that the key to steering the agency is hiring creative managers "who can generate ideas, unfettered sometimes by hard data" to direct its research programmes. "I believe strongly that the best DARPA programme managers must have inside them the desire to be a science-fiction writer," Tether says. H. G. Wells, he adds, would have been good at the job.

And Tether thinks that this approach is working. Today, DARPA "in all of its organizations is banging at the door of highly innovative ideas", he says. "I think anyone who attended DARPATech 2007 in Anaheim would agree." Others aren't so sure that DARPATech is really the best reflection of that, however. The tightly scripted event excludes questions from the audience, and features little detailed discussion of current programmes. Stephen Lukasik, who was DARPA's director from 1971 to 1975, says he worries that DARPATech is now light on substance. "It used to be a technical meeting," he says, where programme managers and attendees had a forum to learn and argue about specific technologies.

And the conference's penchant for Hollywood-style fantasy also underscores the differences between DARPA in the Vietnam era and today. In Vietnam, the Pentagon turned to DARPA to lead counterinsurgency research. But as violence ramped up in the Iraq conflict, it divided up counterinsurgency tasks among other organizations, such as the Joint Improvised Explosive Device Defeat Organization, an office established to fund technologies to defeat the ubiquitous homemade bombs seen in Iraq. Today, DARPA says that the closest thing it has to the Vietnam-era Project Agile

BETTMANN/CORBIS

DARPA's early focus on detecting missile launches has faded somewhat.

is Persistent Operational Surface Surveillance and Engagement, a software system designed to track insurgents by integrating sensor data. Other DARPA technologies are being used in Iraq, such as unmanned aerial vehicles, translation devices and an anti-sniper system.

Privacy issues

DARPA's highest-profile counterterrorism effort, though, proved politically disastrous. After the 11 September 2001 terrorist attacks, Tether — then in his first year of the job — created a new Information Awareness Office under the leadership of John Poindexter, a former national-security adviser and a key figure during the 1980s Iran–Contra scandal. Poindexter and his office proved to be more polarizing than expected; its Total Information Awareness programme (later changed to Terrorism Information Awareness), which aimed to sift through huge amounts of data to track terrorists, was attacked on privacy grounds, and Congress eventually cancelled it. "That was a dishonest misuse of DARPA," says Hans Mark, a former director of defence research and engineering now at the University of Texas at Austin. For its part, DARPA concedes that the office could have been created with more caution, but says that Tether believed that the right technology could have prevented 9/11, and that data mining could prevent similar events in the future.

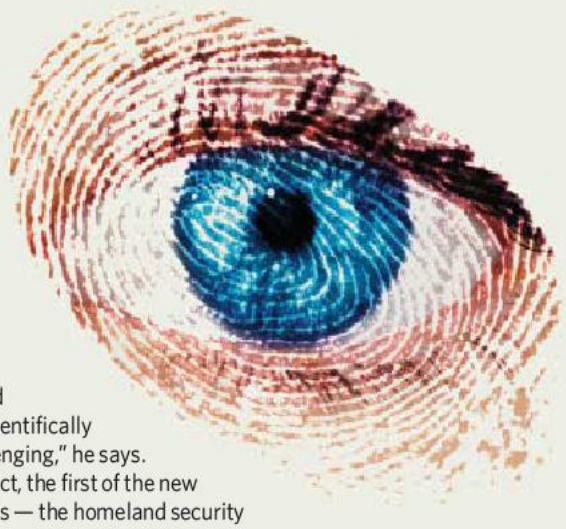
Although former agency directors and senior defence-department officials agree that DARPA is still highly innovative, they don't agree on whether it is as important as it once was to the Pentagon. In one early significant shift at the end of 1969, leaders moved DARPA out of the Pentagon building to make way for Vietnam analysts; DARPA's offices are now located in nearby Arlington, Virginia. Herzfeld calls the move the loss of "a great gift", breaking the immediate link between DARPA and senior Pentagon leadership.

The agency also shifted, in the early 1970s, from issues directed by the president to more self-directed work, although Herb York, one of the agency's founding leaders, notes that the shift was more a product of the time than a change in DARPA's role. "The fact that ARPA moved away from the White House," he says, "is not because of anything ARPA did — it's because of the way the whole scene changed."

Some directors from DARPA's earlier era suggest that later agency directors have tended to choose more conservative projects, leading to less high-impact pay-offs. George Heilmeier focused during his tenure on projects such as radar-evading aircraft and acoustic detection of submarines; the maiden flight of the first stealth aircraft, a project managed and funded by DARPA, took place on Heilmeier's final day as director in 1977. "Then things changed," he says. "Compensation became much less competitive with the outside high-tech world, and DARPA, without the

DARPA: THE NEXT GENERATION

MEHAU KULYK/SPL



At Bolling Air Force Base in Washington DC, Steven Nixon's office is filled with management theory books such as *The First 90 Days: Critical Success Strategies for New Leaders at All Levels* and *The Innovator's Solution: Creating and Sustaining Successful Growth*. Nixon, who is director of science and technology in the Office of the Director of National Intelligence, is studying management culture as part of his plan to do what many people say is impossible: create an organization that replicates the successes of the Defense Advanced Research Projects Agency (DARPA).

In preparing to set up the Intelligence Advanced Research Projects Activity (IARPA), Nixon has consulted with previous DARPA heads, as well as former office directors and programme managers. He thinks that copying the management structure of DARPA will be the best route to IARPA's success — in particular, limiting employees' contracts to four to six years. "That's the key attribute that we plan on borrowing," Nixon says.

IARPA is one of three ARPAs to be proposed or set up since 2002. The others are the Homeland Security Advanced Research Projects Agency (HSARPA) and the newly authorized, but not yet established, Advanced Research Projects for Energy (ARPA-E). Each is meant to bring the sorts of revolutionary breakthroughs made by DARPA to their fields. But it's not clear that a technological solution is what's really needed in each case. Stephen Lukasik, a former DARPA director who was consulted on setting up IARPA, half-jokes that the best model for creating a DARPA-like agency is if an asteroid impact were threatening Earth. "It would be universally understood, the impact would be universally disastrous and it would be scientifically challenging," he says.

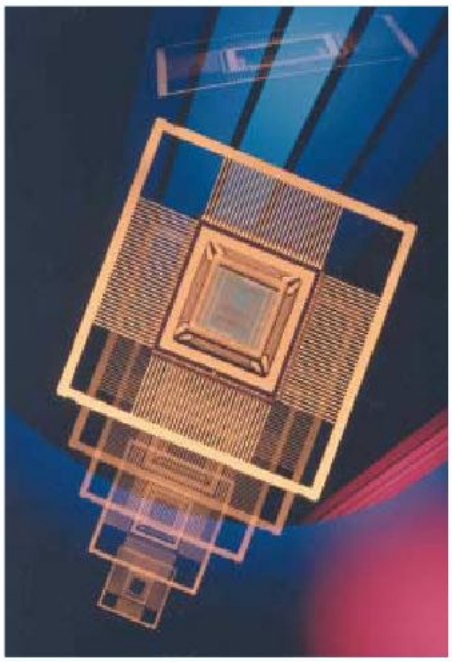
In fact, the first of the new ARPAs — the homeland security one — is already distancing itself somewhat from the original DARPA model. Programme managers at HSARPA do serve limited terms, but so far none of them comes from academia, as many DARPA managers do, says its director Roger McGinnis. The biggest difference, however, is the nature of the projects that HSARPA supports — driven mainly by customers such as the Secret Service and the Transportation Security Administration. "They come to us, we look at the money, and together we choose," says McGinnis. That means that HSARPA tends to focus on specific technologies — for example, miniaturizing biological and chemical sensors to fit in a hand-held device. HSARPA

close connection to the secretary of defence, became more conservative in its selection of new, technology-driven initiatives. To many, DARPA was losing its passion and excitement and seemed to be moving in the direction of a bureaucracy."

While Heilmeier questions DARPA's current commitment to bold research, others accuse the agency of moving away from supporting basic research in universities. In 2005, DARPA became the subject of a congressional hearing to discuss concerns that the agency was cutting off long-term support for universities and, in particular, computer-science departments. "That simply is not correct," Tether told the panel, saying that there had been no decline in support, merely a shift in priorities to interdisciplinary research. One DARPA-supported researcher attributed the change to the director's management style.

"DARPA is the jewel in the defence-department crown." — William Perry

DARPA's leader today doesn't agree that the agency is in decline. Tether points to a host of projects developed in the 1980s and later, such as unmanned aerial vehicles, as well as lesser-known advances such as research on solid-state photon detectors, which enabled night-vision goggles, and integrated circuit research that helped lead to modern mobile phones. Tether also points to miniature global-positioning system receivers and DARPA's funding of gallium arsenide research (for use in semiconductors at a time when the commercial industry viewed such an investment as too risky). Today, he says, nanotechnology is a key research area.



NORTHROP GRUMMAN

Very-high-speed integrated circuits are used in airborne warfare equipment.

Former directors, such as Lukasik and Herzfeld, also credit Tether for starting the Grand Challenge robot races that encouraged younger scientists and engineers to compete for a cash prize to develop a fully autonomous vehicle. The first two competitions were held in the California desert; the third, in November 2007, required the robots to operate in an urban area, obeying traffic laws and avoiding collisions.

Although he hails that accomplishment, Lukasik warns that without presidential challenges, DARPA is in danger of working on problems that are technologically interesting but not important to the nation. "Once you move in that direction," he says, "you move in the direction of more detail, and if that's the case, you run the risk of becoming irrelevant because your measure of survival is political adroitness rather than technical excellence and solving important problems." ■

Sharon Weinberger is a freelance writer in Washington DC.

See Editorial, page 374, and Essay, page 403.

also differs from DARPA in another key area: its budget is $60 million a year rather than $3 billion.

A separate challenge for the ARPA spin-offs is whether they have enough access to key leadership in their areas. "For any organization to have a chance to be as successful as DARPA, it has to report to the very top of the organization," says current DARPA director Anthony Tether. That's not the case at the intelligence or homeland-security ARPAs. "I don't think people understand just how important it is for the director to report directly to the top and have the flexibility to make key decisions and execute bold, new, technology-driven initiatives," adds George Heilmeier, a former DARPA director.

Another issue for the new ARPAs is deciding what to fund. Citing classification issues, Nixon can speak only broadly on this subject, but he points to cyberspace and biology — particularly synthetic biology and biometrics — as key subjects for the intelligence community. "These are areas that are going to be transforming science and will have a huge impact one way or the other on intelligence," he says. Still, he notes, "if we have an intelligence breakthrough, my fervent hope is that people won't know about it, at least for a while."

Nixon, however, does say that IARPA will be a new source of funding for the academic community, calling it the "next big game in town" for basic research. Of course, how much money that will be is hard to quantify—IARPA's budget, like most aspects of the US intelligence budget, is secret. "I would think it's in the hundreds of millions of dollars," speculates Jeffrey Richelson, a fellow at the National Security Archive in Washington DC and a long-time expert on the intelligence community's science and technology programme. Although it might be too early to know how academics will view IARPA, the University of Maryland in College Park has agreed to house it, as part of the agency's bid to foster academic collaboration. And this month, IARPA named Lisa Porter, a well-regarded physicist and senior NASA official, as its first director .

The energy ARPA is proving the most polarizing of the new ARPAs (see *Nature* **447,** 130; 2007). Although the legislation creating the agency says that it is meant to "support revolutionary and transformational energy research where risk and payoffs are high," ARPA-E has no obvious customer. DARPA has the Department of Defense, IARPA has the intelligence community, but ARPA-E faces a complex maze of private-sector industries.

The idea for ARPA-E emerged from a report by the National Academy of Sciences and found support last year in Congress, which passed a bill to create the new agency. But the Bush administration didn't support the proposal, and energy secretary Samuel Bodman expressed concerns about additional bureaucracy and draining money from basic research. Funds for ARPA-E have still not been appropriated, and it's unclear where the money will come from. Martha Krebs, a former head of the Department of Energy's Office of Science, expresses grave doubts about the proposal. "I believe ARPA-E has been born out of a misconception that the defence model can and should work for energy," she says.

Politics, not technology, could eventually prove the downfall of the new ARPAs. DARPA emerged from the extraordinary atmosphere that surrounded Sputnik; six years have passed since the 11 September 2001 terrorist attacks, and the bureaucracy is entrenched, notes John Foster, a former director of defence research and engineering. "The important thing is to recognize what it took to start DARPA and sustain it for 50 years. Do we have those conditions in energy, do we have it in intelligence, in homeland security?" he asks. "No." **S.W.**

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All this week's Correspondence was written in response to the Editorial 'Venezuela's way ahead' (Nature **450**, 922; 2007).

Venezuelan students are campaigning for freedom

SIR — You indicate that President Hugo Chávez has broadly supported universities and scientific research (*Nature* **450**, 922; 2007). But you report that his proposals to bolster his powers and retain his position indefinitely have been met with opposition, spearheaded by protest marches of hundreds of thousands of students and their professors.

As rector of Simón Bolívar University and vice-president of Venezuela's Academy of Physical, Mathematical and Natural Sciences, I disagree with your suggested explanation for this apparent inconsistency, that students in public Venezuelan universities are mostly from the upper middle class.

All students, rich and poor, dislike authoritarian regimes. Student protests were sparked off in May after Chávez ordered the closure of a major television station whose views opposed those of his government. Students demand freedom of speech and — confronted with the president's insistence on imposing a narrow-minded Cuban-style socialist state on Venezuela — freedom of choice. They want universities to stay autonomous and free from government intervention. Their fight is not about left-wing or right-wing politics, but to reconcile opposing and radical views in the interests of their own future.

Benjamin Scharifker
Universidad Simón Bolívar, Apartado 89000, Caracas 1080A, Venezuela, and Academia de Ciencias Físicas, Matemáticas y Naturales, Palacio de las Academias, Avenida Universidad, Caracas 1010, Venezuela

Independent institutes are being financially strangled

SIR — Many of the assumptions made in your Editorial 'Venezuela's way ahead' about government benevolence (*Nature* **450**, 922; 2007) overlook the realities. Although substantial funds are potentially available for research, support is not as widespread or as academically competitive as you imply.

Universities not under direct government control are being financially strangled. One example is the country's largest university, the Central University of Venezuela, whose budget allocation has been unchanged for four years, although the country has the highest inflation in South America (its "rapid economic growth" is mostly the result of massive state spending, supported by an ever-increasing reliance on oil income, not a consequence of real productivity). This year, the university is precluded from hiring new staff and promotions are ruled out.

There is no class struggle at Venezuelan public universities, which are free and have a nationwide entrance exam. Some private institutions give scholarships and levelling courses for underprivileged students. Venezuelan students are simply supporting universal rights such as freedom of expression and academic freedom, and taking a firm stance against political discrimination, coercion and imposition of a single ideology.

Governments distributed competitive research grants before Chávez came to power, but now political criteria are more important and the system is being undermined. Given autonomous universities' failure to elect government-sponsored candidates to governing bodies, the state has resorted to physical, legal and financial harassment. The proposed constitution criticized by the student movement could eventually destroy administrative and academic autonomy.

Referendum results have raised hopes of reconciliation and decreasing political polarization, and of slowing the exodus of talented young Venezuelans.

Name and address supplied. The author is a research professor at a Venezuelan university.

Government control has weakened universities

SIR — Further to your Editorial (*Nature* **450**, 922; 2007), the academic freedom of Venezuela's autonomous universities, where some 90% of all scientific research in the country is carried out, has come under attack by the present government, which has opened a competing and parallel university system of questionable academic quality.

Contrary to what you imply, President Chávez has weakened the universities in his efforts to bring them all under government control. The country's premier research institute, the Venezuelan Institute for Scientific Research in Caracas, for example, is now under the control of the government, which plans to incorporate it into another submissive academic unit. Few departments at Venezuela's 47 universities have the staff and equipment for internationally competitive research.

It is not clear that Venezuelans will be able to "build on some of [the president's] genuine achievements" because many Venezuelans are in doubt about what these achievements are. In my area of expertise, government policies are hurting the meagre but honourable academic productivity of my country.

Orlando Albornoz
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Social sciences worst off as projects remain unfunded

SIR — Your Editorial (*Nature* **450**, 922; 2007) brings welcome attention to Venezuelan science and to the defeat of President Chávez's proposed reforms to allow himself to stay in power indefinitely. But I do not agree with your remarks about resources.

Students are not all from upper-middle-class backgrounds — a social category that Chávez has removed anyway, by quenching private enterprise. For decades, university students have been drawn from the middle and lower classes, and all the more so now, when the Gucci-clad revolutionary leaders claim that nearly a million of the country's 26 million people are studying at university.

The Organic Law of Science, Technology and Innovation (LOCTI) may support some worthwhile projects, but they are funded by compulsory tax deductions that otherwise would go to the Ministry of Science and Technology, largely known for its incapacity to administer anything. Last year, it failed to spend 75% of its budget. Current government policy is to use LOCTI funds to pay science and technology research grants, but hundreds of sound scientific projects — especially in social sciences — go unfunded. During 2006, the only available source of funding for science was the Misión Ciencia, a presidential initiative, as the government did not support other funding opportunities.

Jaime Requena
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Grants awarded on the basis of political criteria

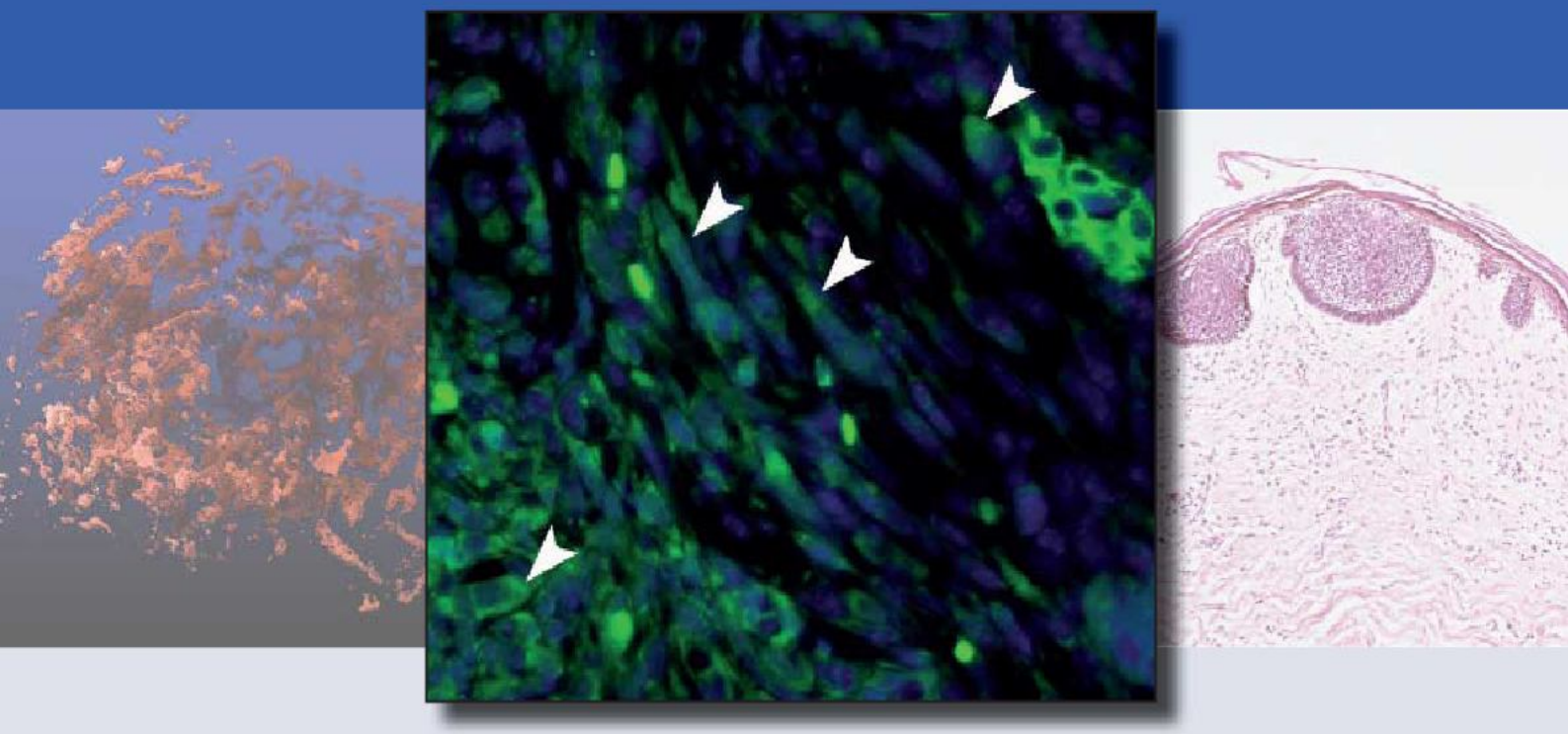
SIR — Your Editorial about Venezuela seems very optimistic (*Nature* **450**, 922; 2007). You point out that the present government created the Ministry of Science and Technology, claiming that this "distributes grant money on a competitive basis".

In fact, the ministry was set up to replace CONICIT, the national council for scientific and technological research: an autonomous institution of almost 30 years' standing that did indeed use peer review and scientific excellence as criteria for distributing grants. The present ministry, however, emphasizes political criteria when assigning grants.

Venezuela's share of scientific publications, as measured by PubMed, fell from 0.054% in 1998 (the year before Chávez became president) to 0.04% in 2006.

Klaus Jaffe
American Center for Strategic Studies, Universidad Simón Bolívar, Apartado 89000, Caracas 1080, Venezuela

Laboratory Investigation



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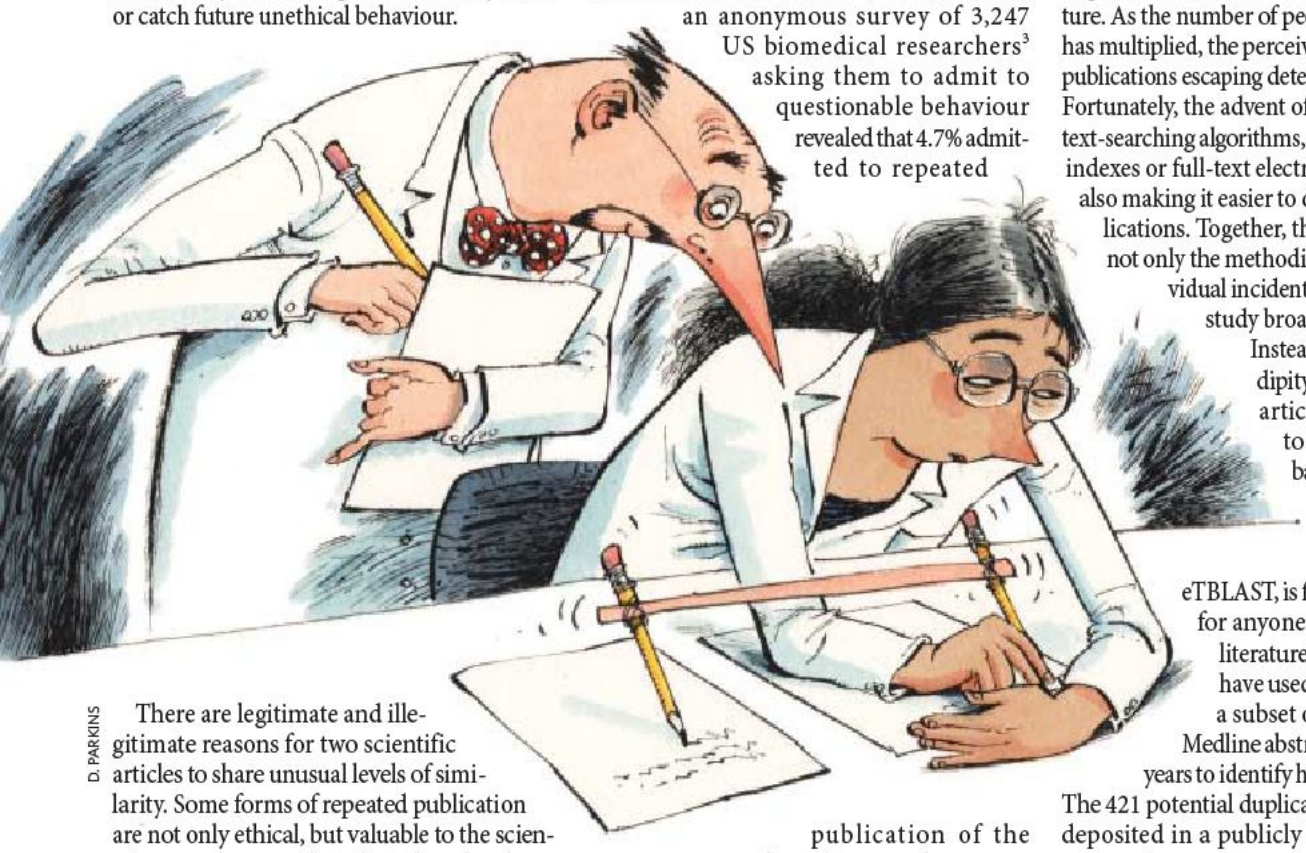
COMMENTARY

A tale of two citations

Are scientists publishing more duplicate papers? An automated search of seven million biomedical abstracts suggests that they are, report **Mounir Errami** and **Harold Garner**.

With apologies to Charles Dickens, in the world of biomedical publications, "It is the best of times, it is the worst of times". Scientific productivity, as measured by scholarly publication rates, is at an all-time high[1]. However, high-profile cases of scientific misconduct remind us that not all those publications are to be trusted — but how many and which papers? Given the pressure to publish, it is important to be aware of the ways in which community standards can be subverted. Our concern here is with the three major sins of modern publishing: duplication, co-submission and plagiarism. It is our belief that without knowing whether these sins are becoming more widespread, the scientific community cannot hope to effectively deter or catch future unethical behaviour.



D. PARKINS

There are legitimate and illegitimate reasons for two scientific articles to share unusual levels of similarity. Some forms of repeated publication are not only ethical, but valuable to the scientific community, such as clinical-trial updates, conference proceedings and errata. The most unethical practices involve substantial reproduction of another study (bringing no novelty to the scientific community) without proper acknowledgement. If such duplicates have different authors, then they may be guilty of plagiarism, whereas papers with overlapping authors may represent self-plagiarism. Simultaneous submission of duplicate articles by the same authors to different journals also violates journal policies.

Previous studies that have tried to gauge the level of unethical publishing have mostly relied on small surveys of specific communities. One of the largest to date used text-matching software to trawl more than 280,000 entries in arXiv, an open-access archive of mathematics, physics, computer science, biology and statistics papers. The study suggested a low number of suspected acts of plagiarism (0.2% of arXiv papers), but a much higher number of suspected duplicates with the same authors[2] (10.5%). In 2002, an anonymous survey of 3,247 US biomedical researchers[3] asking them to admit to questionable behaviour revealed that 4.7% admitted to repeated publication of the same results and 1.4% to plagiarism.

> "The duplication of scientific articles has been largely ignored by the gatekeepers of scientific information."

In general, the duplication of scientific articles has largely been ignored by the gatekeepers of scientific information — the publishers and database curators. Very few journal editors attempt to systematically detect duplicates at the time of submission. The US National Library of Medicine, based in Bethseda, Maryland, curates the primary biomedical citation index, Medline, and currently reports fewer than a thousand cases of duplication since the 1950s, discovered mainly by serendipity. Yet if the results of the anonymous survey[3] are extrapolated to the Medline database (more than 17 million citations and growing steadily), then you would expect to find closer to 800,000 cases. Where between these two vastly different figures does the true number lie?

The academic arms race

Establishing a baseline is a crucial first step, but in our view, monitoring trends is even more important to the health of the scientific literature. As the number of peer-reviewed journals has multiplied, the perceived odds of unethical publications escaping detection have improved. Fortunately, the advent of new computational text-searching algorithms, along with electronic indexes or full-text electronic manuscripts, is also making it easier to detect unethical publications. Together, these advances enable not only the methodical discovery of individual incidents, but also a means to study broad trends.

Instead of relying on serendipity to identify duplicate articles, we have chosen to search online databases, such as Medline, using text-similarity software. The search engine, eTBLAST, is freely available online for anyone to use to search the literature[4]. In recent work, we have used eTBLAST to search a subset of more than 62,000 Medline abstracts from the past 12 years to identify highly similar entries[5]. The 421 potential duplicates found have been deposited in a publicly available database, Déjà vu (http://spore.swmed.edu/dejavu), and after manual inspection were confirmed as duplicates with different authors (0.04%; based on inspection of full-text articles), or duplicates with the same authors (1.35%; based on inspection of the abstracts). The rate of false positives in this study was only 1%. But without full text it may be difficult to determine if suspected duplicates properly attributed the earlier work.

Whether or not the duplications are legitimate papers has yet to be established.

Extrapolating to the subset of Medline records that have abstracts (8.7 million), this would correspond to roughly 117,500 duplicates with the same authors[4]. Although this number is far higher than the 739 records currently annotated as duplicates in Medline, these duplication rates are substantially lower than those found in arXiv, perhaps reflecting differences in the database formats (preprints versus journal papers), or disparities between these fields in what is considered acceptable practice. There is also variation in how these estimates were reached, including the subjective nature of manual inspection (we used two manual checkers in each case). The Medline database, unlike arXiv, is limited to titles and abstracts, and so automated comparison of full-text articles is not possible, perhaps making it harder to detect more sophisticated duplications.

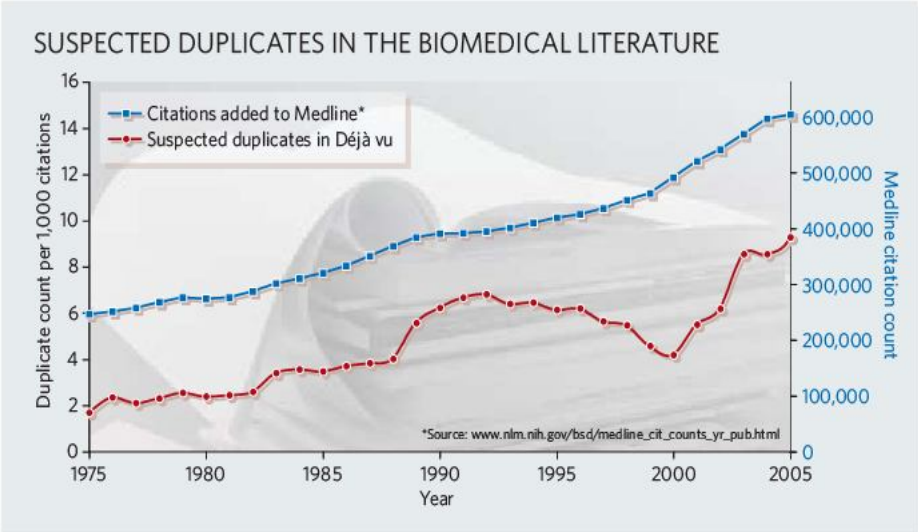


Figure 1. Increasing opportunity? The number of biomedical papers indexed in the citation database, Medline, has grown steadily over the past 30 years. A search of 7 million abstracts, using the text-matching software eTBLAST, reveals tens of thousands of highly similar articles (unpublished data), which are also growing in number. Are these legitimate or illegitimate publications?

Closer than close

Because of the sheer size of the Medline database, scaling up the eTBLAST search to all 17 million records would be extremely time consuming even though each search takes only about 40 seconds. Fortunately, we observed that 73% of the Medline duplicates identified in our initial study and curated in Déjà vu also feature as the 'most related article' in Medline (calculated by a Medline algorithm). So, we downloaded the related abstracts for 7,064,721 Medline records, and compared the original and related abstracts against one another using eTBLAST. This approach allowed us to complete our analysis in 10 days rather than 10 years. In this way we have identified a further 70,458 highly similar records, all of which have been deposited in Déjà vu.

Given the limitations of our process, we expect around 50,000 of these to be true duplicates. This is partly because we used a less stringent duplication threshold for the latest data set and so after manual checking 27% of the records turn out to be false positives (see http://spore.swmed.edu/dejavu/statistics). To date, 2,600 of the Déjà vu records have been manually inspected alongside the original, but until that is done the status of each entry remains unverified. However, extrapolating to the entire database, we estimate there are potentially more than 200,000 duplicates in Medline, after various correction factors have been applied.

Although manual verification of the Déjà vu database is very much a work in progress, and so analysis of the full data set should be interpreted with caution, we have started looking for trends in the approximately 70,000 candidate duplicates. With the articles so far captured within the Déjà vu database, merged with analysis of other data extracted from full-text versions of Medline articles available in PubMed Central (such as publication date, language of article and country of origin), it is possible to begin to identify broad trends in publication behaviour. Perhaps the most obvious is a steady rise in the rate of such publications in the biomedical literature since 1975 (Figure 1).

Medline indexes over 5,000 journals published in the United States and more than 80 other countries worldwide. Rising duplicate publication rates documented in Figure 1 are therefore a global phenomenon. Potential factors contributing to this trend are the explosion in the number of journals with online content (increasing opportunities for unethical copying), and a body of literature growing so fast that the risk of being detected seems to diminish. This last factor may be the most important, and we believe that automated detection processes that can provide an effective deterrent may be our best weapon in fighting duplicate publications.

"Automated text-matching systems are used by high schools and universities. We hold our children up to a higher standard than we do our scientists."

One argument for duplicate publication is to make significant works available to a wider audience, especially in other languages. However, only 20% of manually verified duplicates in Déjà vu are translations into another language. What of the examples of text directly translated with no reference or credit to the original article? Is this justified or acceptable? And is such behaviour more widespread for review-type articles for which greater dissemination may be justified? We do not yet have answers to these questions.

In general, we find that the duplication rate extracted from the total Déjà vu database for each country is roughly proportional to the number of manuscripts that country contributes to Medline (Figure 2). The top eight contributors to Medline are the United States, Japan, Germany, China, the United Kingdom, Italy, France and Canada, representing close to 75% of all Medline records. However, two of these countries, China and Japan, have estimated duplication rates that are roughly twice that expected for the number of publications they contribute to Medline. Perhaps the complexity of translation between different scripts, differences in ethics training and cultural norms contribute to elevated duplication rates in these two countries.

Simultaneous submission

With few exceptions, the repeated publication of the same results by those who conducted the research is ethically questionable. It not only artificially inflates an author's publication record but places an undue burden on journal editors and reviewers, and is expressly forbidden by most journal copyright rules.

Examination of typical submission and publication dates from 10,000 articles randomly selected from PubMed Central, shows that on average the review process takes 4.3 months and that 97% of articles complete this process within 10 months (see Supplementary information). Curiously, as many as one-third of the manually verified duplicate abstracts in Déjà vu sharing at least one author are also published less than

five months after the original. Examination of the submission and publication dates of these pairs confirms that many of these duplicates must have been submitted simultaneously to different journals in violation of journal policies and accepted norms. For instance, the Déjà vu database contains many pairs of highly similar abstracts with overlapping authors that appear in the same month, all apparently acts of simultaneous submission to multiple journals.

Duplication by different authors

Articles sharing excessive similarity with other papers with different authors do not necessarily represent plagiarism, as there are sometimes valid or trivial reasons (such as a simple author name change). However, considering only those duplicates in Déjà vu where the full text of both articles has been manually inspected, we have found 73 plagiarism candidates, most of which were previously undetected. Discerning the difference between legitimate and illegitimate duplication is beyond the capacity of automated algorithms (and apparently many scientists), and so it is critical to withhold judgement of any candidate duplicates until evaluated by a suitable body such as an editorial board or a university ethics committee. As part of our study, we have started to send out requests for additional information for such cases, one of which has initiated an investigation by a journal. It is our intent to send such requests for information to all individuals and journals involved in, or affected by, duplicate records with different authors.

Many duplicate articles without authors in common go undiscovered. Are the perpetrators then likely to repeat the offence? Searching the Déjà vu database reveals several repeat practitioners, and manual inspection of full-text articles confirms some of these as suspected serial offenders. As with any potential illegitimate duplication, caution and careful human judgement must be exercised, and detailed comments and manual assessments for these and other duplicate pairs can be found within the Déjà vu database.

Unlike repeated publication by the same authors, simultaneous publication is rarely observed for duplicates that do not share authors (see Supplementary information), undoubtedly due to the fact that it is usually difficult to re-use someone else's work before it appears in print — unless the duplicating author also happens to have been a referee of the original. Although anecdotes abound of referees stalling a publication in order to give themselves time to duplicate and publish the same result first, the general lack of duplicates with different authors appearing in rapid succession suggests that this is either rarer than feared, or that the perpetrators do a good job of concealing it.

In general, duplicates are often published in journals with lower impact factors (undoubtedly at least in part to minimize the odds of detection) but this does not prevent negative consequences — especially in clinical research. Duplication, particularly of the results of patient trials, can negatively affect the practice of medicine, as it can instill a false sense of confidence regarding the efficacy and safety of new drugs and procedures. There are very good reasons why multiple independent studies are required before a new medical practice makes it into the clinic, and duplicate publication subverts that crucial quality control (not to mention defrauding the original authors and journals).

What can be done?

Although duplicate publication and plagiarism are often discussed, it seems that discussion is not enough. Two important contributing factors are the level of confusion over acceptable publishing behaviour and the perception that there is a high likelihood of escaping detection. The lack of clear standards for what level of text and figure re-use is appropriate (for example in the introduction and methods) is a well known problem; but the belief that one can get away with re-use is probably the single most important factor.

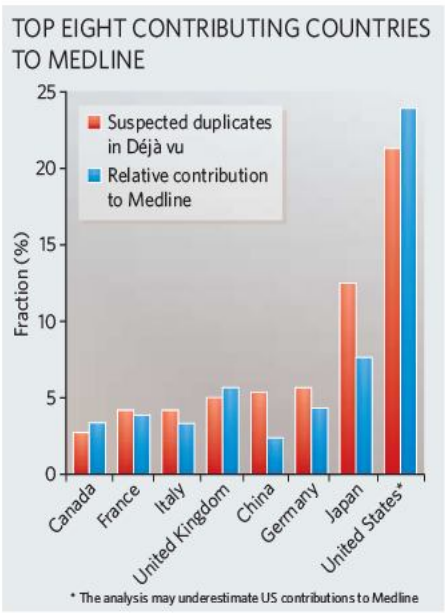


Figure 2. Duplication is a global activity. The proportion of suspected duplicates in the Déjà vu database for each country was estimated (unpublished data) by assigning articles to countries based on the corresponding author's address. Also presented is each country's relative contribution to Medline estimated from 180,000 randomly selected Medline articles.

Addressing these two aspects could be relatively quick and easy. If journal editors were to use more frequently the new computational tools to detect incidents of duplicate publication — and advertise that they will do so — much of the problem is likely to take care of itself. We find it odd that automated text-matching systems are used regularly by high schools and universities, thereby enabling us to hold our children up to a higher standard than we do our scientists. In our view, it would be fairly simple to fold these tools into electronic-manuscript submission systems, making it a ubiquitous aspect of the publication process.

Although text-comparison algorithms have come a long way in the last decade, they are still in their infancy, and experience with student software shows that as tools to detect duplicate publication improve, determined and skilled cheats will find ways to defeat them. But as in any arms race, the winners are usually determined by the cost–benefit balance, and the costs entailed in unethical duplication practices will quickly rise to a level that makes them prohibitively expensive to all but the most desperate (or most skilled) practitioners.

"The fear of having some transgression exposed in a public and embarrassing manner could be a very effective deterrent."

There are additional practical avenues for improving Medline and other databases, such as more aggressive enforcement of copyrights by journals, and the creation of an 'update' publication category under which clinical updates and longitudinal surveys in sociology or psychology could be categorized, and these should be explored.

But above all, the fear of having some transgression exposed in a public and embarrassing manner could be a very effective deterrent. Like Dickens's Ebenezer Scrooge, the spectre of being haunted by publications past may be enough to get unscrupulous scientists to change their ways. ■

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Supplementary information is linked to the online version of this article at www.nature.com/nature

BOOKS & ARTS

Behind the scenes

A Natural History Museum researcher unlocks its cluttered store rooms to expose an extraordinary past.

Dry Store Room No. 1: The Secret Life of the Natural History Museum
Richard Fortey
Fourth Estate/Knopf: 2008. 352pp.
£20/$27.50

Henry Nicholls

I have been inside Dry Store Room No. 1 twice. The fluorescent lighting casts a thin, blue light on a cold room crammed with zoological curiosities — the most precious specimens of London's Natural History Museum.

Several aisles are dedicated to fish. Thousands line up nose to tail as if shoaling along the shelving. One wall is dominated by reptiles: giant tortoises jostle for their postmortem position behind lock and key. A tall glass case holds the head of a giraffe, still attached to its neck like a Victorian lamp to its post.

For Richard Fortey, this seldom-visited vault beneath the dramatic public galleries is "a physical analogy for the jumbled lumber-room of memory". It cradles the facts and recollections that he has stored away during his career as a leading palaeontologist at this great museum. His latest book, *Dry Store Room No. 1*, curates these memories to provide a hugely entertaining social history of the museum.

The Natural History Museum has seen some famous figures, among them nineteenth-century naturalists Richard Owen and Edwin Ray Lankester. Yet Fortey focuses on lesser-known characters, such as the ordinary (sometimes extraordinary) scientists who worked in the institution since it moved to its current site in 1883, when the natural history specimens of the British Museum were relocated to South Kensington.

Many of these people have devoted their working lives to one obscure twig of the tree of life or a single class of minerals. It is hard to contain a sense of bewilderment when introduced to someone for whom the maggots of carnivorous screw-worm flies are "almost like old friends". Yet Fortey champions the importance of nurturing esoteric expertise. Making sense of the world's detail enriches our lives and reminds us what it means to be part of nature's intricate system. Sometimes, seemingly arcane knowledge can save lives. It was familiarity with parasitic screw-worm flies that helped quash a major disease outbreak in Africa that could have brought misery to millions.

Fortey's anecdotes about the scientists themselves paint a vivid picture of the characters that make up this unsung research community. Commendably, he leaves few skeletons in closets. In one case, after the death of a museum botanist who worked on a family of tropical plants in the early twentieth century, his colleagues made quite a find. On clearing his office, they came across a card index of sexual conquests, filed alphabetically, each adorned with a sprig of pubic hair. "Once a curator, always a curator," ventures Fortey.

NHM

A bug's life: beyond the galleries, a researcher catalogues specimens at the Natural History Museum.

As Fortey feeds on this rich human history, something interesting happens: the Natural History Museum comes alive. It appears as a bureaucratic beast evolving in response to selective pressures, such as changes in funding infrastructure, increasing pressure to publish, technological innovation (like DNA-profiling) and the escalating expectations of its public.

The final chapter confronts the evolutionary landscape ahead of the museum. It maps out an intriguing and optimistic future for natural history. A new breed of web-savvy amateur naturalists will pass on findings to professionals, Fortey predicts. The need for these experts "as guardians of the collections and as 'quality control' on the taxonomy produced by the wider community" will become more pressing than ever.

Would I have enjoyed *Dry Store Room No. 1* as much if I'd not had the rare privilege of setting foot inside the eponymous room? If this were not a book about my favourite museum? If I didn't live in London or indeed in Britain? Almost certainly. The research connections are global: Fortey roves from Moroccan mountains in search of trilobites to the shores of Russia's Lake Baikal netting diatoms to Antarctica to collect meteorites. The scientists' stories are gripping, some with the implausible plot of a soap opera, many with the intrigue of a spy novel and others with the humour of a comedy sketch.

Ultimately, the goings-on behind the cathedral-like façade in South Kensington are similar to those taking place at any major museum of natural history and Fortey broadens his conclusion accordingly. The great museums, he says, may be the only places for future generations to find "the answer to the question: What have we done?" This five-star read is a perfect tribute to the cluttered wonder of the real Dry Store Room No. 1. ■

Henry Nicholls is the editor of the journal *Endeavour* and author of *Lonesome George: the Life and Loves of the World's Most Famous Tortoise*.

Networking knowledge

Scholarship in the Digital Age: Information, Infrastructure and the Internet
by Christine L. Borgman
MIT Press: 2007. 336 pp. $35

Richard Akerman

The invisible college is becoming visible as the Internet enhances the ability to exchange scientific information. More open science is enabled by lifting constraints on communication and allowing broader and richer dissemination and discussion of theories and results. Academia and society are running to keep up with the changes in technology and the expanding capacity of scholarly infrastructure. New business models and academic reward systems are also burgeoning.

Scholarship in the Digital Age provides an overview of these transformations from a rigorous historical perspective that is couched in well-defined terminology. As Christine Borgman points out, "This is an opportune moment to think about what sort of scholarly environment we should be building ... while the new technologies receive the most attention, it is the underlying social and policy changes that are most profound."

Borgman focuses on scholarly communications practices and issues, encompassing all domains of science. She presents the academic information system as embedded in and constrained by a complex socio-political environment. Her perspective provides a useful balance to the ambitious and sometimes utopian technology focus of major e-research projects.

To frame the problem, Borgman looks first at the essentials of scientific communication, describing the mechanics of peer-reviewed publication, the range of conference types and contributions by authors. Although well-worn ground for seasoned scientists, this is a good foundation for assessing the impact of the Internet on the dissemination of science.

It is to the disruptive impact of the Internet on science that Borgman turns next, exploring the challenges caused by openly circulating digital information, particularly issues for scientific publishers. Although there is a benefit to society because the closed social networks that used to form invisible colleges of researchers have now opened up, their erudite discussion, painstakingly gathered data and solid research are now sharing space — and competing for search ranking — with materials of questionable quality and legitimacy.

How does one select, register and certify research documents in a networked digital environment? And how can one locate quality articles, particularly when much of the record is available only to licensed users and may be poorly represented in general search results? A detailed discussion of open access ensues, including issues of intellectual property and copyright, as well as commons approaches, such as Creative Commons (http://creativecommons.org), in which content creators may grant a limited set of rights, explicitly permitting use for public purposes.

Borgman then discusses data and all their aspects — from basic definitions and new types to increasing volumes of data and their role in scientific communication. She revisits certification, curation and intellectual-property issues for data, which are subject to different conventions to that of publications.

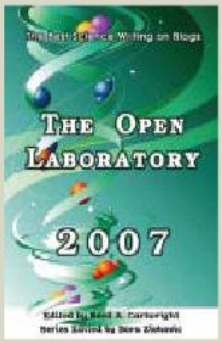
The author sets up her concept of an "advanced scholarly information infrastructure" based on her research and explicitly set within a framework of open science as a public good. In her description of the infrastructure, there is little in the way of technical guidance and the focus is on the behaviour and patterns of different disciplines, the nature of academic artefacts (such as books and journal articles) and on the conventions and etiquette of collaboration. Indeed, the topic of collaboration in various sections of the book — consistent with the non-technical focus — includes no mention of 'wikis' (websites for collaboratively editing and sharing text, such as Wikipedia).

Scholarship in the Digital Age concludes with a compelling discussion of the research problems that a comprehensive scientific cyberinfrastructure will need to address. The author points out the necessity of taking a long-term view of content, to balance local and global requirements. Also, there is a need to separate content from tools and services (thereby creating a 'content layer' that gives a coherent view of all content types), and to identify tools, technologies and institutional mechanisms to balance the coherence and control of the content layer. Thus, it creates a compromise between the desire of scientists for unfettered direct access and the needs of content-owners to control access.

Science policy-makers would do well to refer to this book in framing their aspirations for a scholarly infrastructure. Students of information science, as well as those seeking to understand science in our digital age, will find it a valuable reference and starting point. In a world where scientific networks and communication are now increasingly visible and open, Borgman has illuminated the discussion of the scholarly communication system itself. ■

Richard Akerman is a technology architect at the National Research Council Canada Institute for Scientific and Technical Information, 1200 Montreal Road, Ottawa, Ontario K1A 0R6, Canada.

Parallel lives



The editor of this second anthology of the best scientific communiqués from the blogosphere thinks blogs offer new ways to discuss science. *The Open Laboratory 2007: the Best Science Writing on Blogs* (Lulu.com, 2008) takes the curious approach of using dead tree format to highlight the diversity of scientific ideas, opinions and voices flowing across the Internet. Every year a different guest editor — here Reed Cartwright, a blogger and genetics and bioinformatics postdoc from North Carolina State University — picks the best posts to coincide with the Science Blogging Conference (in North Carolina on 19 January). First-hand accounts bring to life the stresses of a graduate student, a mother returning to the bench and an archaeologist's joy at unearthing mammoth fossils. Topics tackled are as varied as the writers, from Viagra and tapeworms to trepanning. Explanations are often offered with a personal twist, such as a father's tale of his child's Asperger's syndrome. The measured voices of trustworthy academics make medical research easy to swallow. If you are overwhelmed by the surge in science-related blogging and don't know where to start, then this compilation may help you steer a course through the sea of perspectives on offer — or inspire you to start a blog yourself.

Mark Oliver Everett sought to understand the world through music, not science. The lead singer of US rock group Eels made sense of his troubled family history first via song-writing and now in an autobiography — *Things the Grandchildren Should Know* (Little, Brown, 2008). His late father, the physicist Hugh Everett III, suggested the radical idea of parallel worlds in quantum theory — every time a certainty is measured, the universe splits in two parallel strands, each with a different outcome. The breadth of his father's ideas are not lost on Mark, who poignantly links them to his sister's suicide: in one world she may be dead, yet in another alive. It is an intriguing personal perspective on an influential scientist's life from his family and to note the impact his science made on a young man with a powerful messenger, the guitar. J.B.

FILM

Science at Sundance

The 2008 Sundance Film Festival, ending this week in Park City, Utah, included a healthy handful of science-themed films. Since the successes of 2005's feel-good Antarctic birdfest, *March of the Penguins*, and 2006's feel-bad Al Gore lecture *An Inconvenient Truth*, scientific documentaries have been in the ascendant — especially those about the environment.

Among the documentaries angling for a screen at the local multiplex, or at least a slot on television, are two that tackle conservation issues. *Fields of Fuel* is a paean to biodiesel starring the young activist Josh Tickell. *Flow: For the Love of Water* is a global look at how water scarcity drives corruption and big profits for certain corporations, a theme also explored in the science-fiction film *Sleep Dealer*.

Meanwhile, *The Linguists* documents two researchers travelling the globe recording languages that are about to become extinct. "It points out a global crisis that people may have only been vaguely aware of," says one of the pair, David Harrison of Swarthmore College near Philadelphia.

More upbeat fare is to be found in the shorts. A series on the sex lives of insects, collectively titled *Green Porno*, sees Isabella Rossellini — also the director — dressed as a dragonfly (pictured) in the mood for love. Another, artsy short, *Untitled #1*, imagines a strange world in 2507. Creator Nao Bustamante has run a booth at the festival where attendees leave video messages for the future that, she promises, will be lovingly transferred from medium to medium as technology changes in the coming 500 years.

Finally, a hip short film that is actually a preview for a forthcoming flash-based computer game, *Gas Zappers*, spotlights the goofier side of global warming. It depicts a polar bear (what else?) defeating carbon-emitting villains with the help of such figures as Intergovernmental Panel on Climate Change chairman Rajendra Pachauri — who is clad as Moses and uses his tablets to beat back waves threatening to flood Venice. **E.M.**

PREVIEW

Revealing reptiles

Life in Cold Blood
television series written and presented by David Attenborough

Ed Yong

"What do you think this is?" asks David Attenborough, handing me a heavy rock. Sitting in his London home, filled with wildlife paintings and tribal artefacts, I have been drawn into a game of 'guess the fossil'. The specimen turns out to be vertebrae from an ichthyosaur found in his neighbour's garden. "I went over and there was this, just lying about!"

Attenborough is as expected from his on-screen appearances — knowledgeable, eloquent, a consummate storyteller and extremely excited about wildlife. He is as happy enthusing about turtle mating as he is about the grisly habits of the caecilian, a burrowing worm-like amphibian whose young feed by tearing strips of fatty skin from their mothers. The most interesting thing he's eaten himself? "Big moth caterpillars in New Guinea. You put them on a fire and they come out like Twiglets."

The turtle and caecilian sequences feature in *Life in Cold Blood*, his new television series about reptiles and amphibians. "These are the last major classes of terrestrial animals that we haven't given a series to," he explains. The programmes are the culmination of the *Life* series, which began almost 30 years ago with *Life on Earth*. Attenborough says that he wants to make a box set indexing every species and topic covered. "I will be very pleased to be able to put that on a shelf."

Snakes, lizards and frogs might be less of a draw than meerkats and monkeys, but he relishes the challenge. "In a way, it's a great advantage because it means that a lot of their stories haven't been told. In my view, public-service broadcasting shouldn't just be about making programmes about popular animals."

Only once does he pause, when I ask him if a shoot has ever disappointed him. He describes a trip in 1955 to the Aru Islands in Indonesia to film birds of paradise. "We turned up in Jakarta with a camera, didn't speak a word of the language and didn't have a letter of introduction from anybody. It was ridiculous." The government accused them of being spies and refused entry — even Attenborough can be defeated by bureaucracy. "We hastily thought of something else and went off to film Komodo dragons but we didn't get that either. It was hopelessly amateur and cack-handed but quite good fun."

Technology has improved since then, reducing the need for staged sequences. The question of artifice in natural-history programmes flusters Attenborough. He is incensed by an article complaining that a spitting cobra sequence used a captive animal. "I am not making an adventure programme ... I'm trying to demonstrate that there's a snake that squirts [venom] through its fangs like a hypodermic needle."

Attenborough is pragmatic. "I remember one woman who wrote in after watching a lion kill a wildebeest and said that it was absolutely scandalous. It would be much better to [train] lions to eat grass!" Talking of the need for balance: "It would be improper and disgraceful if you just dwelt on the violence and yet, if you don't show it, you are so distorting reality that you are producing fairytales. If people saw what we put on the cutting room floor."

To him, animals are gripping enough without help. "They are unpredictable, very often new, extraordinarily beautiful, dramatic and they share something with us, which is life." He sees his programmes as restoring our childlike fascination with living things, allowing people to "get back in touch, which is essential if they're going to take responsibility for nature".

Attenborough doubts we will arrest the decline in the world's biodiversity. "The thing that really appalls me is that there are three times as many people alive on Earth as when I started making programmes. The space left for other species has been eaten up. Just that is enough to dampen any *joie de vivre* I have."

He is equally modest about his fame. Dismissing his status as the result of "doing it forever and fairly regularly". He recognizes the tougher competition today, noting that an advertisement for a researcher on the *Life of Birds* attracted 3,000 applicants, one-third of whom had doctorates. "I feel almost guilty because I started when nobody wanted to do it."

He regains his spark when talking of his fellow film-makers. "There's no shortage of talent. All one asks is that they treat the animals with respect, and if they treat them with knowledge and admiration then that's a bonus." As for himself, he has no plans to go quietly into the sunset. He is scripting a programme on evolution to tie in with Darwin's bicentennial in 2009. ■

Ed Yong is a freelance science writer based in London. He blogs at http://notexactlyrocketscience.wordpress.com.

A longer version of this story appears on network.nature.com. *Life in Cold Blood* is first broadcast on 4 February (BBC 1); DVD released on 25 February.

How the change agent has changed

As the US military's research arm turns fifty — and other branches of government seek to adopt its famously nimble approach — a former director reflects on what worked and what didn't.

Charles Herzfeld

For the past 50 years the small organization now named the Defense Advanced Research Projects Agency (DARPA) has sought to influence the behemoth that is the US Department of Defense. The services generally walk the evolutionary road; DARPA aims for revolution. It has often succeeded — the $150 billion, in today's dollars, that the United States has spent on the agency's programmes has brought us the Internet, stealth aircraft, precision warfare, seismic systems, lasers and much more.

Being the agency's director was the most challenging and exciting job of my career. I did my best work, with the most important consequences. It was the only post that demanded all my energy, ability and determination, and made it worthwhile to give them. A shining star over my life, the agency also cast deep shadows. With power, responsibility and action came criticism and opposition — some helpful and well-meaning, some not. Lasting friendships and enmities grew. Looking back, I am satisfied with what we achieved, and with the credit that we got — although that was often slow in coming.

Now, plans are afoot to emulate DARPA's approach in fields such as energy, homeland security and intelligence. The time, then, is ripe to reflect on its ups and downs since February 1958.

Big problems

In October 1957, Sputnik surprised the United States. Its launch made President Dwight Eisenhower anxious about US military capability and high-tech research, and eager to reorganize the lumbering and diffuse US missile and space effort. Early in 1958, working with the President's Science Advisory Committee (PSAC) and his science adviser Jim Killian, Eisenhower created the Advanced Research Projects Agency (ARPA; the 'D' was added in 1972). For maximum flexibility and effectiveness, the president gave the agency a small number of big jobs, independent leadership and new funds. It was run from the Office of the Secretary of Defense, separate from the military services, under the directorship of the industrialist Roy Johnson.

ARPA's initial 100 or so staff had three tasks. First the agency was to restructure the missile and space programme, accelerating its most significant parts. Second, ARPA was to drive a new initiative in ballistic-missile defence. Third, it was to launch a programme to detect nuclear explosions in the atmosphere, the ocean, in space and underground.

This was just the beginning. Over the next 5 years, ARPA's other responsibilities and achievements would include reforming research and teaching in materials sciences and engineering — physics, chemistry, metallurgy, ceramics and others — by pursuing important integrations and restructuring. The agency led the development of advanced computers and communications for the defence department's command and control systems. Finally, and less successfully, the agency was tasked with helping the South Vietnamese defence against the North and the Viet Cong guerillas.

US ARMY, 1963

The author (right) in 1963 with Robert Wienecke (centre) and Lt Col Reuter before an inspection trip in South Vietnam.

All these programmes attacked large problems that mixed technical, managerial and conceptual challenges. They sometimes required serious work outside physics and engineering, as in the implications for strategy and arms control of anti-ballistic missile systems and nuclear-test detection. They also necessitated an understanding of unconventional warfare, such as guerilla action and the strategic, linguistic, cultural and historical aspects of counter-insurgency. The army and navy were generally averse to such projects and, more comfortable with well-established techniques, concentrated on marginal improvements.

ARPA pursued a whole-lifecycle approach to invention, originating most of the technical ideas behind its programmes, using the best scientific advice to craft their structure, and working with government organizations and outside contractors to execute and apply the results. This holistic management style is the defining feature of the 'ARPA way', and it continues today.

Big solutions

Before its first year was out, ARPA had reorganized the US missile and space programme. Civilian and scientific space programmes went to a new agency, NASA, that absorbed the old National Advisory Committee for Aeronautics. Control of military missile programmes went to the services that used the weapons, and intelligence programmes went to intelligence agencies. A pattern of 'action before paper' was set by the launch of the US answer to Sputnik, Explorer 1, in January 1958 — a month before ARPA's official start.

The chief architect of ARPA's enduring atmosphere and approach was its third director, Jack Ruina, who ran the agency from 1961 to 1963. Ruina, a brilliant young professor of electrical engineering from the Massachusetts Institute of Technology, was creative, relaxed and witty, yet serious and stimulating. He managed through discussion and suggestion, not memos; he devoted his and our attention to the top issues and the hardest problems; he got the best technical advice; he stressed the importance of leadership within and by ARPA; and he mandated his staff to act quickly.

Specifically, Ruina restructured ARPA's advanced anti-ballistic missiles programme, which he hired me to take over late in 1961. This later provided most of the technology for the Safeguard System, the Strategic Defense Initiative, and the National Ballistic Missile Defense System currently being deployed.

And in 1962, Ruina hired J. C. R. 'Lick' Licklider, the inventor and inspiration of modern universal computing. Arpanet, the precursor to the Internet, was developed during Licklider's two stints as director of the Information Processing Technologies Office, switching on in 1969. Also during the late 1960s, a programme on supercomputers began, starting with the first highly

parallel machine, the ILLIAC IV. My directorship from 1965 to 1967 was a time of consolidation and bridge-building, with Vietnam top of the agenda. I tried to maintain the heady entrepreneurial environment that Ruina created, while pushing for more collaboration with policy-makers, to keep ARPA's funding and its influence healthy.

The effort to support South Vietnam, Project Agile, did not save the country. But in 1963, ARPA gave South Vietnamese troops the lighter and more practical AR-15 rifle. This became the M-16, now the standard US military firearm. A few years later precision warfare began, when the first laser-guided bombs were developed and used in combat. Project Agile also founded the study of counter-insurgency strategy, tactics and philosophy. This helped in northeast Thailand in the 1970s and, unhappily, is relevant once more.

Also in 1963, ARPA launched what came to be called the Interdisciplinary Centers for Materials Science — 12 universities as part of the ARPA programme and 2 universities as part of the Atomic Energy Commission's programme. These interdisciplinary centres, now run by the National Science Foundation, invented the modern approach to materials science, producing much first-rate work. Their many thousands of PhDs and postdocs drove the electronics revolution.

In the 1970s and 1980s I was a member of the Defense Science Board, which advises the defence secretary on top-level matters of military technology; I chaired committees on, among other things, cruise missiles and ocean surveillance, and helped review some DARPA programmes. The 1990s, especially the first Iraq war, was a proving ground for much of the agency's work of previous decades — such as stealth aircraft and precision warfare. Ironically, the same era saw research at the defence department become much narrower in its purview.

Throughout this time, guidance from senior national security figures — especially Johnny Foster (director of defence research and engineering, 1965–72), and several defence secretaries, particularly Bill Perry (1994–97) — helped to provide programmatic continuity.

The art of ARPA

An exemplar of ARPA in action was the programme to detect nuclear tests, one of the agency's great successes of the 1960s and '70s. The schedule was tight, the range of issues enormous, the collaborations numerous, the risks daunting and the payoffs huge. Thrilling stuff.

In 1958, the president asked ARPA to explore whether nuclear explosions could be detected so reliably that the United States might make arms-control agreements, assured that cheats would be caught. Crucially this assignment had the defence secretary's blessing, silencing the inevitable chorus of dissent. ARPA defined the programme's purpose: to build a system that could reliably distinguish natural events, such as earthquakes, solar flares and storms, from nuclear explosions let off underground, in the atmosphere, in space and underwater. These initial steps were primarily about policy, not science, engineering or implementation.

ARPA developed technical concepts for implementing the directive, working with PSAC, the Atomic Energy Commission and others. The work on underground detection alone is an example of the programme's scale. In 1958–59 ARPA began studying underground explosions and earthquakes using 200–300 seismic stations worldwide. By 1963 this showed promise, but more detail was needed. ARPA decided to build an array of connected, sensitive seismometers, and to analyse the data using the beamforming algorithms it had just developed for radar.

DARPA

"Jack Ruina created a heady entrepreneurial environment at ARPA."

Next, ARPA had to come up with one system that cracked all the following problems: improve sensors, learn how best to bury and connect them, collect and clean the signals of noise, develop new computers and computing techniques for beamforming, design a user interface for the output, and explain the results to non-specialists.

The air force took the lead in building and installing the resulting system, with ARPA's help and occasional supervision. To calibrate it, ARPA coordinated two tailored nuclear explosions. Finally the agency oversaw the data analysis and contributed to the policy conclusions. After years of debate on whether the system could distinguish natural and nuclear events, what to improve and how to pay for it, it became apparent that the system gave reliable information on almost all foreign nuclear tests. And the seismic arrays transformed our understanding of Earth's interior.

To be at the heart of this integrative invention, and enjoy it, was truly 'doing ARPA'. An artform.

Lessons learned

In the five decades since ARPA's founding, and the four since my stint at the top, there have, of course, been budget cuts, programme halts, and losses of drive. Relations with the rest of the defence department and services have fluctuated, occasionally approaching crises. There is more oversight and more bureaucracy, and the press have become less friendly.

But one fundamental change to DARPA is more important than all these vicissitudes. In 1958, the body was designed to be an agent for change in the Department of Defense, located in the Office of the Secretary of Defense. In the 1960s, it became stronger and more effective in this role. Sometime in the 1970s or '80s, the agency shrank to being a agent for change in the Office of the Under Secretary of Defense for Acquisition, Technology and Logistics, which focuses on building and buying weapons. This marginalization went with a downgrading of the remit of the Director of Defense and Engineering, and the shrinking influence of science on US policy generally.

Yet DARPA is still the most vibrant, impactful organization in defence science and technology in the United States and probably the world. Current director Anthony Tether still leads the way in space technology, the applications of quantum physics and precision warfare. He has spearheaded several new fields, notably the application of neuroscience and electronics to war-zone prosthetics, which should have enormous civilian consequences. Tether has also returned to ARPA's roots with projects on supercomputing, and autonomous ground-vehicle navigation, again with many potential applications.

There are lessons from all this for today's DARPA and those trying to replicate it. Some previous imitators have failed because their goals were too small or leash too short; some had weak leadership or no new money. If you want to build a real agent for change, build yourself a real DARPA. Give it very hard, broad problems to work on. Hire the brightest leaders, problem-solvers and risk-takers, and give them significant resources, guidance, encouragement and freedom. Establish an open, demanding and easy style, with adequate turnover. Hire staff who will build, find more good people, and move on. Then let them work, let them succeed or fail, and give lots of praise. Finally, defend them against friendly fire, for there will be lots. ■

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See also pages 374 and 390.

NEWS & VIEWS

CARBON CYCLE

Harvest of the century

Emilio Mayorga

A century-long record of levels of inorganic carbon in the Mississippi, extracted from the water-treatment plants of New Orleans, documents the changes wrought by shifting agricultural practices in the river's basin.

Concerns about the effects of increasing levels of atmospheric carbon dioxide on climate and ocean acidity have intensified the study of how carbon is exchanged between the atmosphere, land and oceans — especially how CO_2 is removed from the atmosphere and transferred into long-term storage (sinks). The rates of removal into terrestrial and ocean sinks seem to be slowing down[1], highlighting the need for improved data on the individual sinks, their controls and likely future behaviour, and their relationship to other carbon fluxes.

In the global carbon cycle, rivers are a fundamental, multi-faceted link between atmosphere, land and ocean[2,3]. Five years ago, a study[3] of the Mississippi River in the United States found a large increase in the export of dissolved inorganic carbon to the ocean during the past 50 years, a result of the enhanced dissolution (weathering) of rock minerals. Links to changing agricultural practices and crop management, as well as to increased precipitation, were found, but the mechanisms remained unclear. On page 449 of this issue, Raymond *et al.*[4] revisit this topic using a remarkable, century-long data set and more robust approaches. They conclude that direct effects from changes in land use outweigh the indirect influence of climate change.

The chemical weathering of rock material in soils typically consumes CO_2 of atmospheric origin through reactions with carbonate and silicate minerals. These reactions are controlled by factors including climate, mineral type, the availability of acids (particularly carbonic acid) and physical erosion[5]. The resulting bicarbonate ion (HCO_3^-) is the dominant form of dissolved inorganic carbon in most rivers, and does not exchange readily with atmospheric CO_2. Instead, it is transported by water run-off to rivers and, ultimately, to the ocean, where it contributes to the ocean's alkalinity — and so its capacity to absorb CO_2 and buffer changes in acidity.

The weathering of silicate rocks such as basalt and granite results in complete conversion of consumed CO_2 to bicarbonate in soils and groundwater, and the weathering of carbonates such as limestone converts one molecule of mineral CO_2 to bicarbonate for each molecule of atmospheric CO_2 consumed[5]. Overall, chemical weathering is a sink of CO_2 on land, and represents a transfer of atmospheric carbon and buffering capacity to the ocean at timescales relevant to human activity (hundreds to thousands of years). But in all this, human alterations of weathering fluxes through land management had typically been neglected in large-scale assessments.

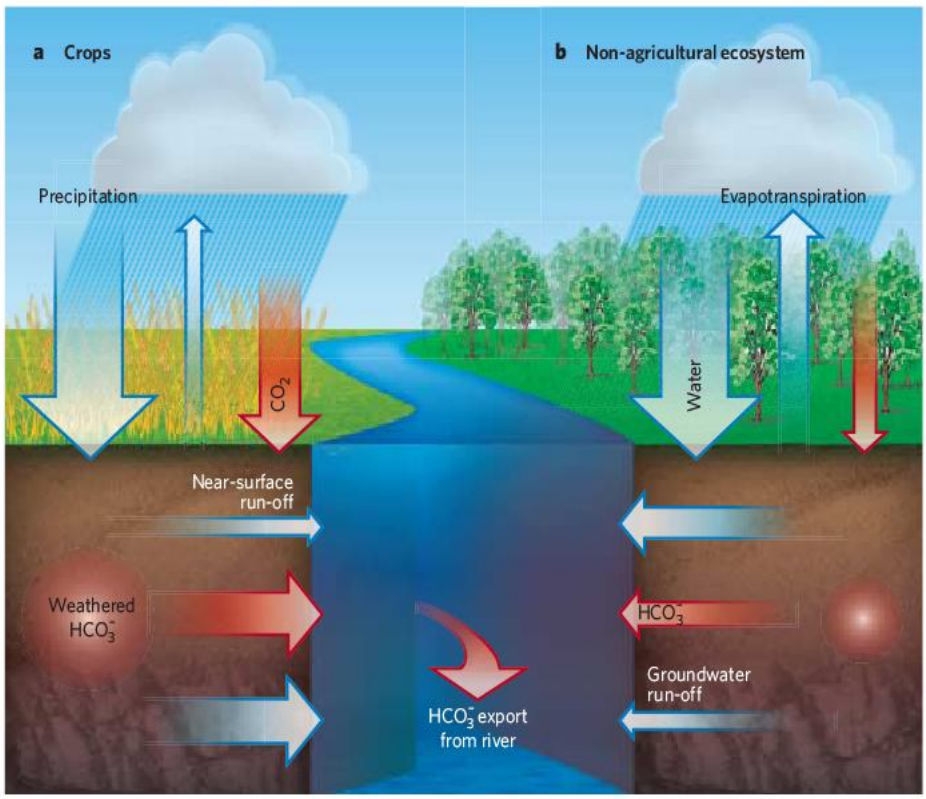

Figure 1 | Carbon exchange and land-use change. Even with similar soil types and well-buffered (mineral-rich) bedrock material, water and CO_2 fluxes through soils in cropland (**a**) and non-agricultural ecosystems (**b**) can be very different. For the same precipitation rate, certain agricultural conditions (including drainage systems, crop type and tillage practice) result in a greater fraction of annual water flows occurring as groundwater run-off and a lower fraction occurring as evapotranspiration. The increase in groundwater run-off results in larger export to rivers of bicarbonate (HCO_3^-) derived from the consumption of atmospheric CO_2 during chemical weathering, as demonstrated by Raymond and colleagues' 100-year Mississippi time series[4]. Agricultural practices such as lime application might also increase the concentration of bicarbonate in soils and groundwater.

Raymond *et al.*[4] exploit alkalinity measurements taken at the water-treatment plants that supply the city of New Orleans with drinking water drawn from the Mississippi. Combined with information about river discharge from the US Geological Survey, these data provide an extraordinary 100-year record of monthly variability in water and bicarbonate export from North America's largest river. They show little change before the 1950s, but confirm the approximately 40% increase in bicarbonate export over the past 50 years previously assessed using an independent record[3].

To investigate the factors driving this increased annual flux, Raymond *et al.* separate out two possible mechanisms. First, there are increases in water discharge that increase total amounts of bicarbonate export, but imply no change in bicarbonate concentration.

Second, there are increases in bicarbonate flux at equivalent discharge values, representing net increases in mean bicarbonate concentration. The authors assess this second effect by normalizing annual bicarbonate flux to the long-term mean annual water discharge. They conclude that more than 60% of the increased bicarbonate flux results from increased concentrations, and not mainly from increased water discharge as was previously thought[3].

Alterations in how the watersheds of the Mississippi basin work seem to have driven the increased bicarbonate discharges of the past 50 years. This period coincides with agricultural intensification, and changes in crop type and land-management practices in the basin. One must bear in mind, however, that the data from New Orleans, near the mouths of the Mississippi, provide only a highly aggregated picture. So Raymond *et al.*[4] turn to an extensive sub-watershed data set of bicarbonate discharge, land use and precipitation distribution to probe the role of cropland extent, changes in hydrology and increased precipitation.

They find that increases in discharge in agricultural — but not grassland or forested — watersheds outpaced increases in precipitation. This conclusion agrees with recent hydrological studies[6] that found that agricultural changes in the Mississippi basin, especially the expansion of soya-bean row crops, might be reducing evapotranspiration and increasing the fraction of precipitation that reaches the groundwater and flows to streams (Fig. 1). Together with practices such as liming soil to neutralize acidity, this enhanced flow through deeper mineral-rich soils results in increased bicarbonate exports from agricultural areas. The authors suggest that more than 50% of the total increase in bicarbonate export in the past 50 years results from the direct effect of land-use changes. The influence of precipitation increase and CO_2 fertilization from atmospheric CO_2 increase is comparatively small. Agriculturally driven hydrological alterations will, however, magnify the effect of precipitation increases.

Does enhanced weathering tip croplands into becoming a net sink of atmospheric CO_2 in comparison with the situation in pristine ecosystems such as forests or grasslands? The answer is complex. First, the acceleration of weathering reported by Raymond *et al.*[4] is probably limited to mineral-rich soils and particular agricultural practices. More importantly, other direct human pressures must be considered. Besides the removal of biomass during conversion from pristine ecosystems, agricultural activities commonly lead to enhanced erosion of soils and loss of associated organic carbon, providing a potential source of CO_2 to the atmosphere through oxidation downstream[2]. Sedimentation in the reservoirs that are often built to support irrigation can, on the other hand, serve as a long-term sink for eroded-soil organic carbon[2]. Consequently, the net global effect of agricultural soil erosion on atmospheric CO_2 remains contentious[2,7]. Land-management effects on river-carbon exports should be considered in parallel with the more extensively studied effects on nutrient exports. The Mississippi basin represents a notable example of both[8].

An intensification of groundwater flow and bicarbonate flux analogous to, but of smaller magnitude than, that highlighted by Raymond *et al.* seems to be occurring in some Arctic basins. Here, it is an effect of climate change on freeze–thaw cycles and permafrost coverage[9]. Thus, changes in chemical weathering caused both by direct human alterations and by the indirect effects of climate change seem to be a more significant component of regional and global perturbations of the carbon cycle than was thought.

This latest study[4] adds to mounting evidence that the levels of carbon export from developed watersheds today might not be representative of conditions before pressures such as intensive agriculture and acid deposition from industrial emissions became widespread[10]. Long biogeochemical time-series such as that from the New Orleans water-treatment stations are invaluable tools for discerning what the dominant controls on river-carbon exports are, and how they respond to large-scale human activities. ■

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HIV/AIDS

Virus kept on a leash

Heinrich G. Göttlinger

Without its Vpu protein, the AIDS-associated virus HIV-1 becomes stuck to the surface of the human cell in which it has replicated. The mysterious factor that tethers HIV-1 is probably a cell-membrane protein.

Mammals have evolved various mechanisms to thwart the spread of intracellular pathogens. Thus, even if the infectious agent breaches the first lines of defence, the infected cells express restriction factors that suppress its further dissemination. One such restriction factor prevents HIV-1 from leaving infected cells and is counteracted by an accessory viral protein called Vpu. On page 425 of this issue, Neil and colleagues[1] characterize this factor and provide tantalizing clues about how it functions*.

To escape the infected cell, HIV-1 buds through the cell surface (thus becoming wrapped in an envelope derived from the host cell membrane) (Fig. 1a) and pinches off. But even if the virus reaches this stage of release, Vpu-deficient HIV-1 particles tend to remain trapped at the cell surface. What keeps them there has been a mystery.

The effect of Vpu on virus release has been perplexing, as it seems to be unrelated to the protein's other function of reducing cellular levels of CD4, the HIV-1 receptor on T cells of the immune system. Also, Vpu's effect on release does not seem to require other HIV-1 components, and its importance for virus release varies widely among cell lines.

*This News & Views article and the paper concerned[1] were published online on 16 January 2008.

It has emerged[2,3] that Vpu counteracts a human antiviral factor that suppresses HIV-1 release by tethering mature viral particles to the cell surface after they have completely pinched off. Such retention of HIV-1 particles at the surface of infected cells can also be induced by human interferon-α, a protein that 'jump-starts' a cell's antiviral defences[4]. Moreover, in an earlier study[5], Neil and colleagues found that Vpu counteracts the effect of interferon-α on HIV-1 release.

The authors now identify[1] an interferon-α-induced human protein that fulfils all the criteria for the tethering factor antagonized by Vpu. The protein, which they aptly name tetherin, is expressed only by cells that require Vpu for HIV-1 release. Decreasing tetherin levels in cells that normally produce this protein allows the release of Vpu-deficient viruses. Furthermore, tetherin expression in cells that normally lack this protein selectively inhibits the release of Vpu-deficient HIV-1. Neil and colleagues' findings suggest that tetherin is highly potent, with only minute quantities being enough to efficiently inhibit HIV-1 lacking Vpu.

How can tetherin entrap outgoing viral particles with such efficiency? Little is known about this small protein, but one aspect is clear — both ends of tetherin are inserted in the cell

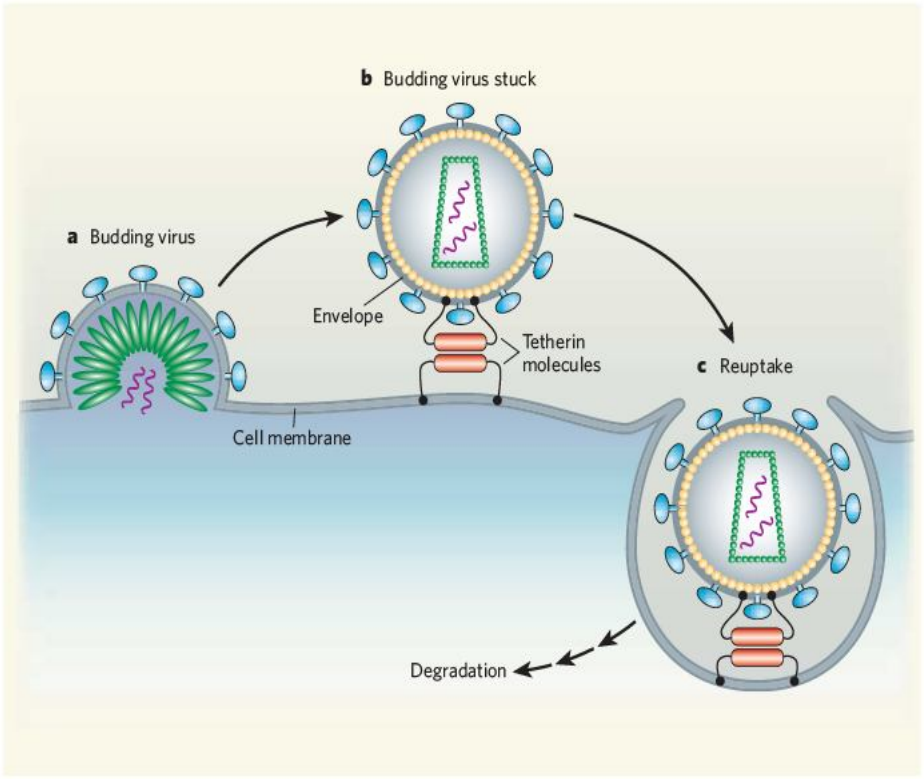

Figure 1 | Model for tetherin-mediated HIV-1 retention. **a**, HIV-1 virions assemble at the cell surface and leave infected cells by budding off from the cell membrane. Assembling virions thus become covered by a cell-membrane-derived envelope. **b**, Tetherin is localized on the outside of the cell surface, but is anchored in the cell membrane at both ends. Identifying tetherin as the cellular factor that prevents HIV-1 release, Neil *et al.*[1] speculate that this protein is also taken up by the budding virion and is firmly anchored in the viral envelope. Virion- and cell-associated tetherin could then interact, preventing the release of the mature virion from the cell surface. **c**, The authors also propose that, through one of its membrane anchors, tetherin might connect to the cell's endocytotic machinery, which engulfs extracellular material in cell-membrane invaginations and imports it into the cell. This could lead to the reuptake of mature virions by infected cells and their subsequent degradation by the cell's digestive system.

membrane through its unusual pair of membrane anchors[6]. The central portion of the protein faces the outside of the cell[6] and seems to interact with the same region of another tetherin molecule[7]. Thus, assuming that tetherin is incorporated into the membrane enveloping Vpu-deficient HIV-1 particles, Neil *et al.*[1] envisage a situation in which tetherin molecules that end up in the viral envelope hold the virus back by interacting with tetherins that are associated with the cell surface (Fig. 1b). Tetherin also interacts with the cell's endocytotic — or internalization — machinery[8], which might play a part in the reuptake of the trapped viruses into the cell and their degradation in intracellular compartments[3] (Fig. 1c).

How does Vpu counteract the effects of tetherin? Neil *et al.*[1] did not detect reduced tetherin levels in the presence of Vpu, although this could have been due to experimental overexpression of tetherin. A previous study[9], however, found that levels of the protein now identified as tetherin are reduced by an entirely unrelated human virus, Kaposi's sarcoma-associated herpesvirus (a finding that also hints at the broad antiviral activity of this protein.) The viral protein responsible in this case, K5, is a ubiquitin ligase enzyme, which adds the molecular tag ubiquitin to proteins, marking them for degradation. K5 is structurally similar to a family of human ubiquitin ligases, at least one of which can strongly reduce the cellular levels of tetherin.

With what turns out to be remarkable foresight, the authors of this earlier study[9] also tested Vpu and found that it, too, decreases the normal cellular levels of tetherin. These observations raise the possibility that Vpu uses a cellular ubiquitin ligase to dispose of tetherin, as it does for CD4. But other possibilities, such as tetherin relocalization by Vpu, rather than its degradation, are also possible.

Only HIV-1 and a handful of its cousin viruses make Vpu, which poses the question of how other related viruses deal with tetherin. For HIV-2 (the less virulent human AIDS virus), a protein that mainly facilitates viral entry substitutes for Vpu, promoting virus release[10], and we will probably soon learn whether this protein also antagonizes tetherin.

Because the amino-acid sequence of tetherin differs considerably among mammals, some HIV-1-related animal viruses might find it difficult to overcome human tetherin, preventing them from becoming human viruses. Conversely, it is worth investigating whether tetherin contributes to the inability of HIV-1 to efficiently escape from most rodent

50 YEARS AGO

"Symmetry of snow crystals" — Despite an infinite variety in the patterns which appear, there does often exist a remarkable symmetry in the six rays [of snowflakes]... Since each of the six arms of the crystal would appear to be growing independently, this symmetry poses a problem in crystal growth, for it almost seems as if each arm of the six-ray star 'knows' what the other five are doing and follows suit... I conjecture that the crystal is vibrating mechanically as a flat plate, in fact as a Chladni plate, with of course a hexagonal symmetry... When the molecules adhere to achieve growth at any particular region on one arm, this immediately introduces a localized damping action on the vibrations... But this very damping is at once felt simultaneously at the corresponding positions on the other five arms. Thus molecules arrive and adhere easily at the other five arms in precisely the same situations as on the first arm; in other words, what happens on any one arm tends to be repeated on the others...
From *Nature* 25 January 1958.

100 YEARS AGO

The product of the world's gold mines for the year 1906 could all be packed in a room 10 feet square and 9 feet high... The value of this 90 cubic feet of gold was nearly eighty-one and a half millions sterling, and its weight nearly 674 tons... Eighty-three per cent. of the total output was secured by the Anglo-Saxon world. According to calculations and estimates made in 1900 by the director of the United States mint, the gold taken from the mines of the world since the discovery of America has amounted in quantity to about 21,424 tons... Nineteen per cent., or nearly one-fifth of the whole, has been mined in the last ten years, and nearly 30 per cent. in the last twenty years.
From *Nature* 23 January 1908.

50 & 100 YEARS AGO

cells[11], which has hampered efforts to develop small-animal models of HIV-1 infection. Even in human cells, Vpu might not always be able to overcome the powerful effect of tetherin, as the release of infectious Vpu-positive HIV-1 can be inhibited with high doses of interferon-α (ref. 5). Thus, an understanding of how tetherin works, and how Vpu fends it off, could lead to strategies to limit the spread of HIV-1 and other viruses that target humans. ■

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MATERIALS SCIENCE

Lilliputian light sticks

Melissa Fardy and Peidong Yang

Building two different fluorescing dyes into a composite organic nanocrystal makes a tunable light generator. At just the right dye proportions, a low-cost, highly efficient source of white light is the result.

If you have ever wondered what makes that ghostly colour in the light sticks used by trick-or-treaters on a Hallowe'en night, wonder no more: a typical answer might be a fluorescing organic molecule such as 5,6,11,12-tetraphenyltetracene, also known as rubrene. Writing in *Advanced Materials*, Zhao *et al.*[1] describe how they used such organic molecules to make white-light-emitting composite nanocrystals that are visible only under a microscope. Cute as these lilliputian light sticks are, they might also point the way to a new generation of light sources.

A typical light stick contains two chemicals: hydrogen peroxide and a phenyl oxalate ester. When these two substances are mixed, energy is released. That energy excites a suitable fluorescent dye, causing it to emit a photon. The wavelength of the photon, and so the colour of the emitted light, depends on the structure of the dye (Fig. 1a). Rubrene, for example, is an orange-emitter; the related molecule 1,3,5-triphenyl-2-pyrazoline (TPP) is blue.

Zhao *et al.*[1] use a process called physical vapour deposition, which is a common way of making organic nanocrystals, to co-evaporate rubrene and TPP at 200–300 °C and condense them onto a substrate at a lower temperature. The result is a collection of uniform organic nanorods with diameters of hundreds of nanometres and lengths of several micrometres (Fig. 1b). Each nanorod functions as a tiny light stick, with its colour determined by the molecular ratio of the two organic dyes.

The energy-transfer mechanism in these nanorods is known as intermolecular fluorescence resonance energy transfer (IFRET), and is fundamentally different from that of a typical light stick. In a typical IFRET process[2], energy is absorbed by one fluorescent molecule (blue-emitting TPP in Zhao and colleagues' case), and transferred non-radiatively to another fluorescent molecule (orange-emitting rubrene). Consequently, the emission of the first molecule is quenched, and the emission of the second is enhanced. The efficiency of this process depends sensitively on the degree of electronic coupling between the two molecules. In an amorphous thin film, for example, where TPP and rubrene molecules are intimately mixed, the emission of TPP can be almost completely quenched with a very small amount of rubrene.

The nanorods prepared by Zhao *et al.* show an intermediate degree of IFRET owing to an unusual structural feature: as X-ray diffraction studies reveal, the rubrene nanocrystals are uniformly embedded in a crystalline TPP matrix. This results in incomplete quenching of the blue emission from TPP, even with decent levels of rubrene 'doping', leading to colour mixing of the orange and blue emissions. Importantly, at the proper molecular ratio, it becomes possible to generate stable white light.

Among researchers investigating comparable lighting devices based on inorganic semiconductors, this kind of colour tunability is often achieved by using homogeneous mixtures of different compounds. A good example is recent research into indium gallium nitride (InGaN) materials, which are considered excellent candidates for solid-state lighting applications. Here, a mixture of indium nitride and gallium nitride is used to systematically shift the emission of the materials from ultraviolet wavelengths to the near-infrared[3]. Similarly, doping has commonly been used in organic light-emitting diodes (OLEDs), both to tune their emission colour and to improve their luminescence efficiency[4].

Many of these doping studies have used amorphous thin films, in which charge carriers have low mobility. Zhao and colleagues' composite organic nanorods not only represent an unusual source of stable white light, but, because of their ordered crystalline natures, should offer better transport properties, and hence better optoelectronic performance. Single crystalline nanowires of the aromatic hydrocarbon hexathiapentacene have been shown to have charge-carrier mobilities almost ten times those of more disordered thin-film structures[5]. To sound a note of caution, however, the emission efficiency of these composite nanorods has yet to be determined. Their integration into a functional electroluminescent device must also be demonstrated.

Because of the great tunability of both their crystal and their electronic structures, inorganic semiconductor nanowires have proved to be workhorses of nanoscale science and engineering[6], finding applications in various electronic, photonic and sensing devices.

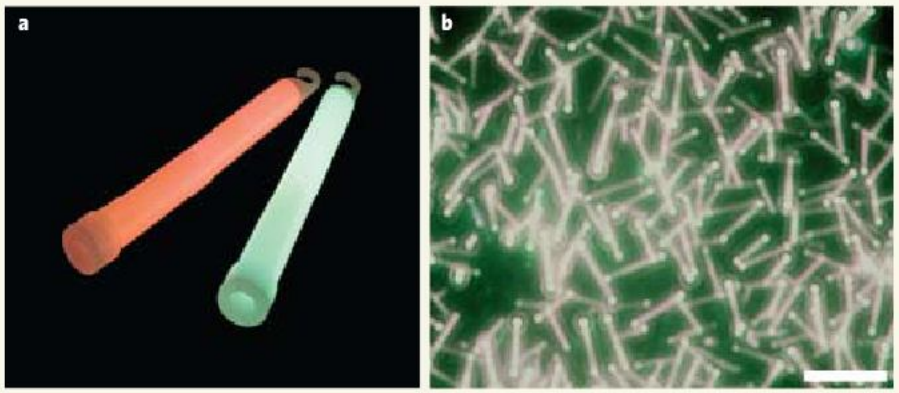

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Figure 1 | Great white hope. **a**, Commercial light sticks containing fluorescent dyes are available in various colours. **b**, Zhao *et al.*[1] engineer similar rods on a tiny scale (scale bar, 5 μm), incorporating two dyes, TPP and rubrene, so that the rods emit white light.

Considering the vast number of optically and electronically active organic molecules, the organic version of the nanowires could reasonably be expected to have similar potential — and indeed, they are already being considered for devices such as transistors, LEDs and photovoltaic cells. Their one-dimensional nanostructures offer the additional advantage of being mechanically flexible, making them particularly appealing for flexible optoelectronic applications: a hexathiapentacene nanowire transistor, for instance, suffers no significant loss in performance when placed under mechanical stress[5].

Just as with their inorganic counterparts, several important optical properties have already been demonstrated in organic nanowires, including lasing[7], waveguiding[8], nonlinear optical mixing[9] and polarized emission[10]. With Zhao *et al.*[1] adding colour tunability to the list, the nanowires seem to have a bright, white future ahead. ■

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PLANETARY SCIENCE

Under Jupiter's pulsing skin

Kunio M. Sayanagi

Fast jet streams blow along the hallmark coloured bands that engirdle Jupiter's surface. By observing how storms erupt in these jet streams and disturb them, we can penetrate deeper into what lies beneath.

Just as physicians use outward signs to diagnose the conditions inside their patients, so planetary scientists get beneath the skin of their equally dynamic subjects by looking at their surface appearances. Penetrating deep into Jupiter, the Solar System's largest planet, is especially difficult, as a thick cloud-deck keeps the lower levels of the atmosphere hidden from telescopic observations. Thus, Sánchez-Lavega *et al.* (page 437 of this issue)[1] study the behaviour of giant storms at Jupiter's visible surface to reveal the vertical wind and temperature structure in the atmosphere beneath.

The vivid stripes of Jupiter make the planet one of the most visually appealing objects in our Solar System. Observations by NASA's two Voyager spacecraft, which flew past in 1979, revealed that these bands of cloud are associated with atmospheric jet streams[2]. These jet streams are remarkably stable compared with their counterparts on Earth, which continually change course and vary in speed. Jupiter's jet streams, by contrast, flow essentially along lines of constant latitude. Measurements made by the Cassini probe[3], passing in 2000 on its way to Saturn, show no change in the jets' large-scale structure since the Voyager observations, and only minor differences in their locations and peak speeds.

Below this thick deck of cloud, however, observational records become scarce. The only direct measurements so far were made by the Galileo mission's entry probe[4,5] in 1995. This probe was designed to reach 140 kilometres below the clouds, but in fact entered a small, anomalously cloud-free region. Applying the information gleaned from this single *in situ* measurement to the rest of the planet is therefore not straightforward.

As part of a campaign of observations to coincide with the passing of NASA's Pluto mission New Horizons, which swung by Jupiter in early 2007, Sánchez-Lavega *et al.*[1] used the Hubble Space Telescope to monitor the Jupiter system. By chance, in late March 2007 they captured the onset of two large convective storms in a jovian jet stream at a latitude of 23° N. This jet stream is particularly notable because it is Jupiter's fastest, with a speed that has varied between 140 and 180 metres per second in recent years[6–8].

The authors monitored the development of the storms using various ground-based telescopes as well as Hubble. The motion of the clouds during the outbursts gives useful hints about the atmospheric structures that lie beneath. In analysing their images, the authors found that the convective plumes substantially overshot the tropopause, a horizontal boundary in the atmosphere located above most clouds that generally acts as a stable dynamical lid on weather phenomena. The storm plumes must be extremely energetic, as they seem to extend more than 100 kilometres vertically from the storm's base below the thick cloud-deck. Towers of cumulus cloud on Earth extend up to only about 10 kilometres or so in the vertical.

To explore this observation further, Sánchez-Lavega *et al.*[1] performed numerical simulations with various background thermal stratifications. They show that, for a storm to become as energetic as those observed, the temperatures below the tropopause must be 2–5 kelvin colder than those measured by the Cassini probe on its 2000 fly-by[9]. It is as yet unclear whether this difference represents a local spatial variance or a change in the vertical temperature structure throughout the latitude band over time. But constraining the thermodynamic

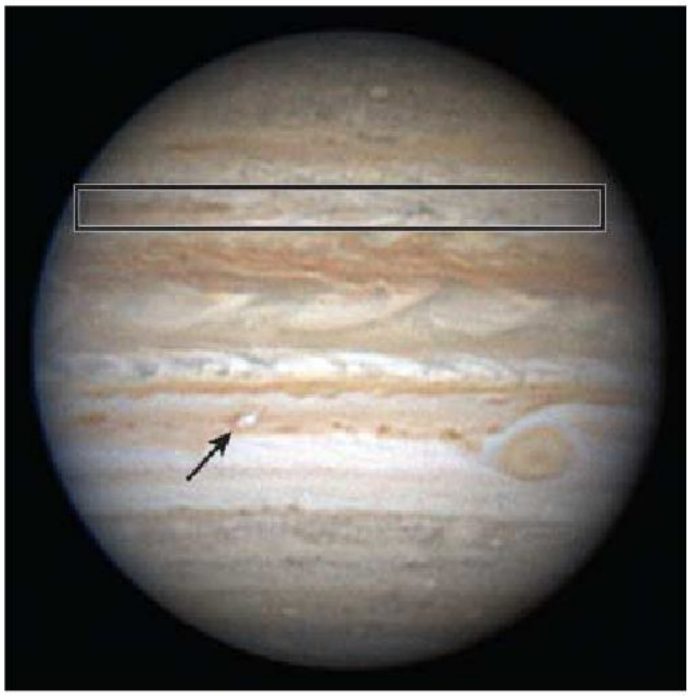

Figure 1 | Storm on camera. An image of Jupiter, obtained on 17 May 2007 from the island of Cebu in the Philippines by an amateur astronomer, shows the planet at the height of its global upheaval of that year. The box indicates the region of the 23° N jet, with the dark grey patches inside it representing the remnants of the disturbance triggered by the storms studied by Sánchez-Lavega and colleagues[1]. The bright spot indicated by the arrow is the onset of yet another storm. The frequency and flexibility of amateur observations, coupled with the ability to achieve spatial resolutions high enough to resolve many major atmospheric features, has made them an integral part of Jupiter observation campaigns. (Image courtesy of C. Go[1,13].)

conditions that allow episodic stormy outbursts is in itself a significant advance.

The authors' analysis also shows that the jet at 23° N remained robust throughout the storm disturbances. Using another numerical model, Sánchez-Lavega *et al.* tested various shapes of vertical shear below the clouds to reproduce the observed jet behaviour, and concluded that the jet stream's speed must increase with depth. This conclusion reaffirms an earlier result[10] reached following studies of a very similar disturbance in 1990.

The disturbance in the 23° N jet marked the climax of a global upheaval on Jupiter, with changes in the colours of the visible clouds in several different latitude bands being seen throughout the 2007 observation season. This turmoil followed a very similar pattern to events in 1975 and 1990 (ref. 11). It is tempting to draw an analogy to a beating pulse — albeit an irregular one, and of unknown origin. It will be interesting to see whether a similar phenomenon returns sometime after 2020, following another break of a similar length. Saturn, too, has recurring giant convective outbursts[12]; both Jupiter and Saturn radiate more heat to space than they receive from the Sun, with cumulus convection transporting a great deal of heat vertically in their troposphere. The pulsing nature of the giant storms on Jupiter and Saturn might even help us to understand how the heat flows from the deep interior to the surface on these planets.

A remarkable aspect of Sánchez-Lavega and colleagues' report[1] is the increasing part now being played by amateur astronomers in planetary observation campaigns. The almost continuous observational coverage by amateurs around the globe allowed the growth and motion of individual large-scale storm clouds to be followed for up to 45 days, while the Hubble telescope performed occasional tracking of fine-scale features for about 10 hours at a time. In recent years, inexpensive digital cameras and access to sophisticated image-processing techniques that correct for the blurring caused by Earth's atmosphere have enabled advanced amateurs with modest-sized telescopes to image planets with resolutions high enough to resolve many atmospheric phenomena (Fig. 1). This coverage from around the world nicely complements the more powerful, but less flexible capabilities of the large ground- and space-based telescopes. Undoubtedly, as the planetary physicians continue to probe deeper under the skin of their subjects, the dedicated amateur medic will be on hand to supply some of the diagnostic tools. ■

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MATERIALS SCIENCE

Designer pores made easy

Michael J. Zaworotko

Imagine being able to tweak the properties of a compound simply by replacing a molecular 'cartridge' with a different one. Just such a capability has been developed in a new class of porous crystalline materials.

Porous materials are creating quite a stir among materials scientists because of their many possible uses, which range from gas storage to drug delivery. But before certain applications are possible, a simple way must be found of tweaking the properties of these molecular sponges. Reporting in the *Journal of the American Chemical Society*, Kawano *et al.*[1] describe just such a method. They have prepared a porous material consisting of two molecular components, one of which can be easily replaced, rather like changing the cartridge in a pen. This flexibility allows the absorption properties of the solid to be fine-tuned.

Metal–organic materials are porous compounds consisting of metals or metal clusters bound to organic molecules known as ligands. Research into these compounds has undergone two bursts of growth over the past couple of decades. The first occurred after the publication of a seminal paper[2] in 1990, which outlined the design opportunities these compounds present for controlling the arrangements of atoms in solids. The second burst occurred in the late 1990s, when it became clear that such compounds combine unprecedented levels of porosity with properties such as magnetism, catalysis, polarity and luminescence[3–5]. This makes them potentially useful for many applications, including for gas storage and separation, as chemical and biological sensors, and even for harvesting energy from light.

Metal–organic materials now deservedly lie at the forefront of advanced materials, offering a synergistic suite of features that gives them several advantages over other porous compounds. Their first useful property is ease of design. Most crystal structures are unpredictable, but those of metal–organic materials are controllable. They also have structural blueprints similar to those found in nature. For example, they can exist as 'zero-dimensional' nanostructures based on atomic polyhedra, rather like those

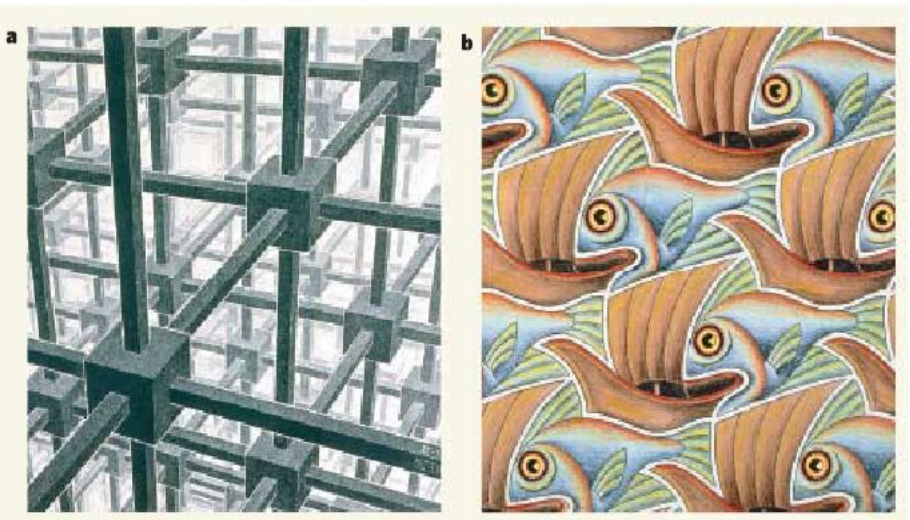

Figure 1 | Blueprints for form and function in porous solids. Metal–organic materials are porous crystalline solids, aspects of which are depicted in these paintings by M. C. Escher. **a**, *Cubic Space Division* (1952) shows a 'cubic net' framework. Some metal–organic materials adopt this structure, in which each cube is replaced with an appropriate molecular building-block. **b**, In *Symmetry E72 (Fish and Boats)* (1949), the spaces between the boats match the shape of the fish exactly. Similarly, metal–organic materials can be designed so that their pores fit exactly around specific target molecules — a prerequisite for molecular recognition.

found in viruses or the carbon molecule buckminsterfullerene. Other metal–organic materials adopt two- and three-dimensional atomic frameworks that have topologies comparable to those of minerals[6,7]. One such topology, known as the cubic net, inspired the Dutch artist M. C. Escher (Fig. 1a). Aesthetic qualities aside, the cubic net is a good example of the framework of a metal–organic material — a modular structure in which the dimensions of the pores are controlled by the size of its molecular building-blocks[6].

The second desirable aspect of these materials is that a given topology can be constructed from very different molecular building-blocks — anything from simple metal ions to complex organic molecules. This means that a desired structural framework can be reproduced at different scales, and with diverse chemical or physical properties. For example, the cubic net can be constructed at an atomic scale from octahedral complexes of metal ions[3], or at 10 to 30 times atomic scale from pseudo-octahedral nanostructures[8]. Furthermore, because these materials often self-assemble from existing molecular building-blocks, they can frequently be prepared in simple, single-step procedures.

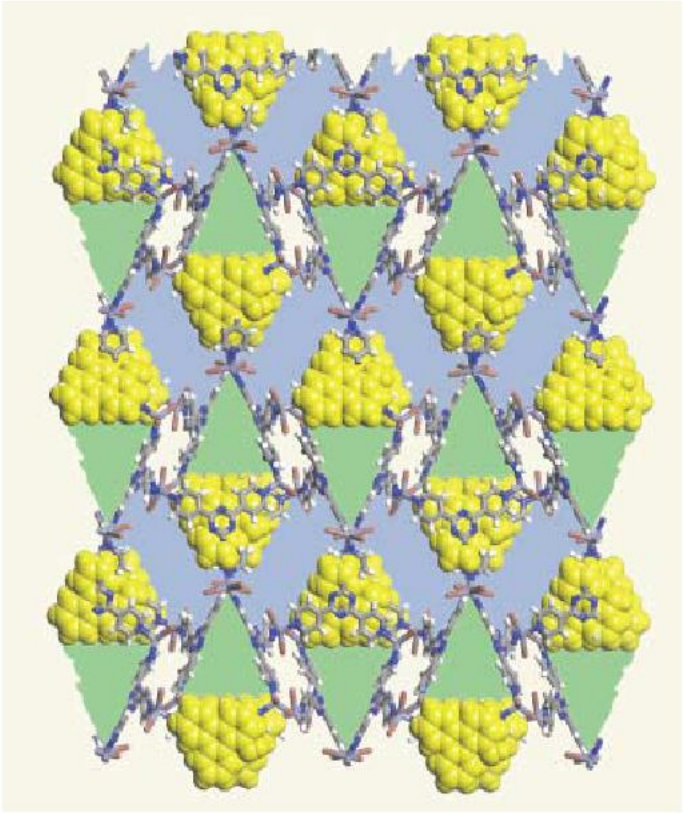

Figure 2 | Modifying the binding properties of porous materials. Kawano *et al.*[1] have made a porous material in which a framework of metal complexes (stick representation) traps bulky organic molecules known as triphenylenes (yellow). The resulting crystal structure forms two types of channel (indicated by the green and blue shaded areas) that can recognize and bind small molecules. Which molecules are trapped depends on the chemical groups attached to the triphenylenes. The recognition properties of the channels can be fine-tuned simply by replacing the triphenylenes with others that have different chemical groups attached.

Finally, the general properties of metal–organic materials set them apart from other porous materials: they are readily characterized, crystalline compounds with well-defined compositions and an unprecedented range of surface areas (as a result of their controllable pore and cavity sizes). Combined with the diverse range of specific properties that can be obtained by varying the molecular building-blocks, these compounds provide a platform of materials that can be adapted for many applications.

An exciting strategy for fine-tuning the properties of metal–organic materials would be to incorporate sites for molecular recognition into their pores, so creating systems that mimic molecular binding in nature. This general concept has also been illustrated by Escher (Fig. 1b). But building molecular recognition into these compounds requires chemical modification of their walls and cavities, to introduce groups that can bind to target molecules (by hydrogen bonding for example). This has, in fact, already been accomplished in a limited fashion using two approaches: pre-synthetic modification, in which chemical groups are incorporated into a molecular building-block before the metal–organic material is synthesized[9], and post-synthetic modification, in which chemical reactions take place in the pre-assembled compound[10,11]. But these are not generally applicable strategies, because pre-synthetic modification of the molecular building-blocks can interfere with the reactions needed to form a metal–organic material, whereas post-synthetic modification often requires harsh reaction conditions that might damage the compound.

Kawano *et al.*[1] describe an innovative solution to this problem — they use interchangeable molecular 'cartridges' to modify the internal surfaces of the compounds. The authors prepared a porous system in which an organic ligand donates electrons to zinc iodide, forming so-called coordination bonds. The resulting network traps aromatic molecules (known as triphenylenes) via non-covalent interactions. This results in a three-dimensional compound held together by a synergistic combination of coordination bonds and non-covalent bonds, and which contains two types of nanopore (Fig. 2).

The triphenylenes can be thought of as molecular cartridges, because they can be replaced during synthesis with other triphenylenes that have different chemical groups attached. Because these molecules form some of the walls of the pores, the pores' molecular-recognition properties depend on which cartridge is present. More specifically, recognition depends on the chemical groups attached to the cartridges. So, for example, one of the triphenylenes used by the authors directs a phenol group — an OH group attached to a benzene ring — towards the interior of the pores; the resulting metal–organic material selectively adsorbs alcohol molecules such as propan-2-ol.

In effect, the authors have found a simple way of fine-tuning the hydrogen-bonding capabilities of their metal–organic material. This breakthrough is not just of scientific interest, as such compounds are serious candidates for membrane materials that will separate alcohols from mixtures of liquids. This property could be useful in biofuel production, for example, or in highly selective chemical sensors.

Kawano and colleagues' discovery realizes long-held aspirations of many physicists. Almost 50 years ago, Richard Feynman had this to say[12]: "What would the properties of materials be if we could really arrange the atoms the way we want them? They would be very interesting to investigate theoretically. I can't see exactly what would happen, but I can hardly doubt that when we have some control of the arrangement of things on a small scale we will get an enormously greater range of possible properties that substances can have, and of different things that we can do."

Feynman would undoubtedly have been thrilled at the possibilities opened up by the authors' molecular cartridges. ■

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GENOMICS

Fighting fire with fire

Daniel F. Voytas

Mobile genetic elements called transposons could cause havoc in the genome if left unregulated. Of the various cellular defence strategies used to preserve genome integrity, one involves exploiting transposons themselves.

The college sophomores in my genetics class become visibly uncomfortable during my lectures on transposable elements. "You mean to tell me there are things jumping around my genome?" they ask when I explain that much of their genetic material is made up of DNA segments that can move about their chromosomes. The mobility of transposable elements is understandably disconcerting, because it would be expected to lead to chromosome rearrangements and mutations that could cause disease. In the long term, however, these elements are probably beneficial, serving as a powerful force for genome change and evolution.

Although the long-term view may do little to appease a 20-year-old, my students can take some comfort in the findings of Cam *et al.*[1] reported in this issue (page 431). The authors show that, in the fission yeast *Schizosaccharomyces pombe*, proteins called CENP-Bs are drafted in to quell transposon activity. What is unusual about this particular choice is that CENP-Bs themselves are derived from transposable elements.

CENP-Bs were first discovered because they bind to repetitive DNA sequences called centromeres[2]. During cell division, centromeres assemble proteins to serve as anchor points for the spindle fibres that move chromosomes to the daughter cells. Fission yeast has three closely related CENP-Bs that bind to centromeres and that are crucial for centromere function[3].

Cam *et al.* wanted to know whether CENP-Bs also associate with genomic regions other than centromeres. They found that, indeed, CENP-Bs occur at dispersed DNA repeats, particularly at the Tf2 transposable elements that litter the *S. pombe* genome. The authors show that CENP-Bs bind to Tf2 and recruit enzymes known as histone deacetylases, which turn off Tf2 expression and prevent transposition. The silenced Tf2 elements are then packaged into subdomains of the nucleus called Tf bodies (Fig. 1).

But transposition can on rare occasions be advantageous, particularly in times of stress, as it has the ability to create potentially beneficial genetic variants[4]. When Cam and colleagues exposed *S. pombe* cells to oxidative stress, Tf2 elements became unbundled from Tf bodies and were expressed (Fig. 1). Furthermore, in cells lacking certain CENP-Bs, the Tf2-related element Tf1 was mobilized, making new insertions that were then packaged into Tf bodies. These observations indicate that CENP-Bs regulate Tf activity through selective packaging.

The CENP-B proteins are an unexpected choice for combating mobile elements because they originate from transposase[5], the enzyme necessary for cutting and pasting DNA during transposition. The transposase progenitor of CENP-Bs belongs to a class of mobile element that is unrelated to Tf2. The authors argue that this unrelatedness suggests a conflict between different classes of mobile element, but this view is probably unnecessarily provocative. As transposases normally bind to DNA, they are suited to being moulded over time into genome regulators. In the case of CENP-B, the vestigial DNA-binding domain of the transposase recognizes centromeric repeats and Tf2 elements. So, whereas CENP-Bs can no longer cut and paste DNA, they can recruit histone deacetylases to regulate Tf2. Other transposase-derived proteins also act as gene regulators. For example, two transcriptional activators used by plants in their response to light are derived from transposases[6], and it is speculated that many such transposase-derived proteins serve crucial roles in gene regulation[7].

Vertebrates also have CENP-Bs, which, like their *S. pombe* counterparts, bind to centromeres, and were only recently shown[8] to contribute to centromere function. The vertebrate proteins also act on centromeric DNA repeats integrated on chromosomes outside the centromere. At these non-centromeric sites, the repeats become tightly packaged into transcriptionally inert protein–DNA complexes called heterochromatin in a way that is analogous to the action of CENP-Bs on the Tf2 elements of *S. pombe*. It is not known whether CENP-Bs also regulate the activity of vertebrate transposable elements, or how the different roles of CENP-Bs in centromere formation and heterochromatin assembly are assigned. Nonetheless, this emerging story suggests a general role for CENP-Bs in helping the cell to manage its burden of repetitive DNA.

The packaging of transposable elements into transcriptionally inactive chromatin is not a defensive strategy limited to CENP-Bs. RNA interference also serves as a surveillance mechanism for double-stranded RNAs that result from the transcription of clustered or scrambled arrays of transposable elements[9]. This process leads to the degradation of messenger RNA transcripts of mobile elements, thus diminishing their activity. RNA interference also assembles mobile elements into heterochromatin, similar to the action of CENP-Bs on centromeric repeats and transposable elements.

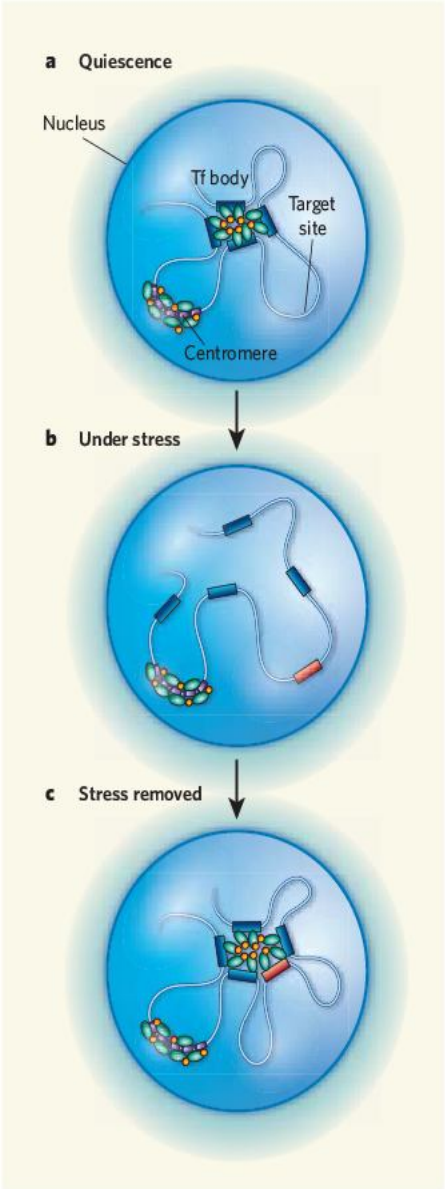

Figure 1 | Under the watchful eyes of CENP-B proteins. **a**, Cam *et al.*[1] show that, as well as binding to centromeric DNA repeats (purple), CENP-Bs (green) bind to interspersed Tf elements (blue). CENP-Bs thus promote clustering of Tf elements into Tf bodies and recruit histone deacetylases (orange) that prevent the transcription of Tf elements. **b**, In rare instances, such as exposure to oxidative stress, the Tf body disassembles, Tf-element expression is activated, and Tf elements can become mobile and transpose to new chromosomal locations (red). **c**, Once the stress subsides, newly transposed and preexisting Tf elements are packaged into Tf bodies.

Of course, the war between transposable elements and their host genome has been going on for millennia, allowing defensive strategies to assume other roles. The RNA-interference machinery in *S. pombe*, for example, has only a modest impact on Tf2 expression[10], yet it is essential for the formation of heterochromatin at centromeric repeats, and is thus important for centromere function[11]. Also, a particularly remarkable finding of Cam *et al.*[1] is that CENP-Bs regulate the expression of some genes adjacent to sites of Tf2 insertion. This indicates that CENP-Bs and transposable elements collaborate to create genetic diversity,

which could benefit the cell. The relationship between transposable elements and their host cell is made more complex by combative strategies that affect regular cellular functions. Undoubtedly, the future holds more surprises about the conflicts and collaborations between mobile elements and their host cell that contribute to genome function. ■

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OPTICS

Against the spread of the light

Kishan Dholakia

Light that propagates without spreading or diffracting sounds like a theorist's pipedream. But it is a very real proposition, and could be used to illuminate some profound aspects of wave–particle duality.

The law of wave diffraction is a tough nut to crack. Consider, for example, a beam from a simple laser pointer. It might still seem narrow and pencil-like when you shine it on a wall or screen a few metres from you, but if you were to point it at the Moon, it would have a diameter of several hundred kilometres. But there are ways to beat the system, and one of them is investigated by Siviloglou *et al.*[1], writing in *Physical Review Letters*. They have generated objects known as Airy wave packets that are entirely resistant to spreading or diffraction.

To appreciate the context of this advance, we must go back more than 100 years, to the time when it was becoming ever more apparent that classical mechanics and classical optics had significant shortcomings. Isaac Newton had originally proposed the idea that light came in discrete packets, which he called 'corpuscles'. But the wave-like behaviour of many optical phenomena swayed scientists towards the wave description of light.

With the advent of quantum mechanics, however, came the notion of wave–particle duality, which pops up in many different guises in physics. In this context, the concept of a wave packet takes on a pivotal, unifying significance. The wave packet is an 'envelope' containing an arbitrary number of waveforms, and also describes the probability that the particle or particles in a certain state will be measured to have a particular position and momentum. Owing to dispersion — waves of different frequencies travelling at different speeds — this wave packet should not remain unaltered. More generally, a given wave packet would then spread or diffract as it moves, changing its shape.

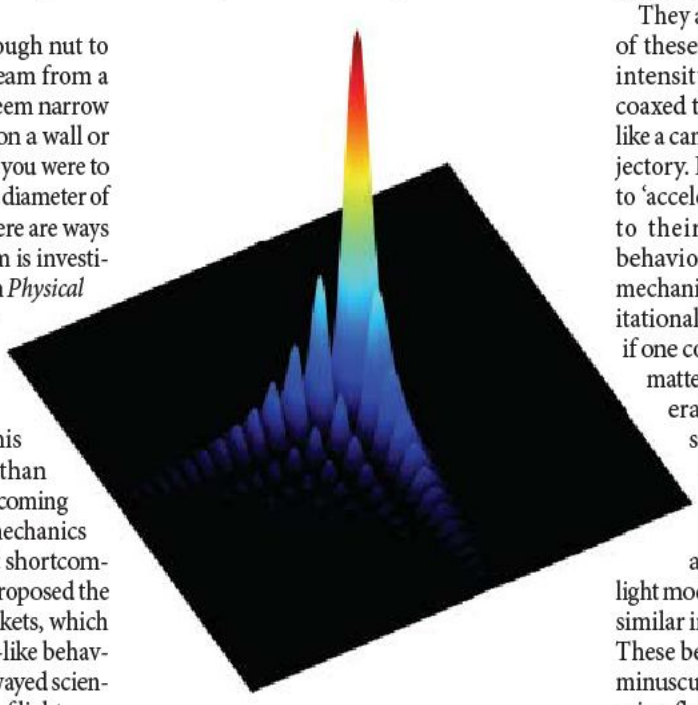

Figure 1 | Upping the Airy — a schematic rendering of the Airy light packets generated by Siviloglou and colleagues[1].

In quantum mechanics, the wave packet is the mathematical solution to the Schrödinger equation, which describes how a system evolves in time. The elegance of the physics is illustrated in the similarity between the mathematical form of the Schrödinger equation (for particles) and the equation describing the diffraction of light. Light offers a very powerful way to explore such wave packets and their evolution, and to make telling analogies to the wave-like behaviour of matter.

The non-diffracting Airy wave packets generated by Siviloglou *et al.*[1] were predicted by Michael Berry and Nandor Balazs almost 30 years ago[2]. They are named after the nineteenth-century British astronomer George Biddell Airy, whose Airy function describes mathematically the way in which a star — an 'ideal' point source of light — is seen in a telescope. Owing to light's wave nature and the telescope's limited aperture, the ideal point image becomes a series of concentric ripples of bright and dark regions. In another demonstration of the underlying connections between matter and light, the Airy function is also the solution to Schrödinger's equation for a particle confined in a triangular-shaped potential well.

Siviloglou and colleagues[1] shone a beam of visible light on a device known as a spatial light modulator. This is an array of liquid-crystal droplets that can present a varying path length to the wavefront of the incident light. Its effect is to mould the wavefront to a desired shape or function. The authors programmed it to produce an Airy function (Fig. 1) and looked at the beam's propagation characteristics. They confirmed that, as had been predicted[2], the intensity profile of a beam of this shape remained unchanged ('propagation invariant') as the beam moved through free space.

They also verified other intriguing aspects of these beams, such as the ability of local intensity maxima in the Airy beam to be coaxed to perform ballistic dynamics, rather like a cannonball, and to follow a parabolic trajectory. Features within the beams were seen to 'accelerate' by starting to move transverse to their direction of propagation. This behaviour may mimic that of a quantum-mechanical particle placed in a constant gravitational field[3]. It would be interesting to see if one could generate such wave packets with matter waves, perhaps in a 'quantum degenerate gas' in which most atoms have settled into a ground state, creating a coherent matter wave.

In the absence of spreading or diffraction in light or matter, exciting applications become possible. Other light modes, such as Bessel beams[4], have shown similar intriguing diffraction-free properties. These beams are now used to exert forces on minuscule particles (such as biological cells) in microfluidic environments, and to achieve targeted drug delivery into cells using ultrashort bursts of non-diffracting light[5], with no precise focusing required. Stopping the spread of light or matter is a theorist's dream, but one with eminently practical consequences. ■

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Q&A

MOLECULAR BIOLOGY

The expanding world of small RNAs

Helge Großhans and Witold Filipowicz

Molecular cell biology has long been dominated by a protein-centric view. But the emergence of small, non-coding RNAs challenges this perception. These plentiful RNAs regulate gene expression at different levels, and have essential roles in health and disease.

How many classes of RNA have been identified?

There are three main types of 'classic' RNA: messenger RNA, transfer RNA and ribosomal RNA. mRNAs are translated into proteins, whereas tRNAs and rRNAs have housekeeping roles during mRNA translation. Small RNAs are not translated into proteins. Instead, these 20–30-nucleotide sequences regulate various biological processes, often by interfering with mRNA translation. Small RNAs come in different forms (Box 1), the best-understood classes being small interfering RNAs (siRNAs), microRNAs (miRNAs) and Piwi-associated RNAs (piRNAs).

When were small RNAs discovered?

During the past two to three decades, researchers identified many small regulatory RNAs, ranging in size from roughly 70 to 300 nucleotides, in various organisms. But the 20–30-nucleotide small regulatory RNAs were discovered more recently because, owing to their small size, they are easily missed in biochemical analysis, and they are poor targets for inactivation by classical genetic tools. Moreover, many miRNAs can compensate for each other's function, which makes their identification on the basis of the overt consequences of their absence difficult. The first two miRNAs to be discovered, *lin-4* and *let-7*, were identified in the 1990s through genetic experiments in the worm *Caenorhabditis elegans*. Then siRNAs were identified in animals, plants and fungi as the effector molecules that mediate the process of sequence-specific gene silencing, or RNA interference (RNAi), in response to double-stranded RNA (dsRNA; Fig. 1). The discovery of RNAi earned its two lead researchers, Andrew Fire and Craig Mello, the 2006 Nobel Prize in Physiology or Medicine.

Have we identified all small RNAs?

Hardly. Although hundreds of small RNAs have been identified in plants and animals, high-throughput sequencing and sophisticated bioinformatics tools repeatedly reveal the existence of new miRNAs and siRNAs. The human genome could encode more than a thousand miRNAs, the equivalent of a few per cent of protein-coding genes. Moreover, new classes of small RNAs continue to be discovered.

Do all organisms have small RNAs?

With the notable exception of the budding yeast *Saccharomyces cerevisiae*, almost all eukaryotic organisms (the cells of which contain a nucleus) investigated so far have siRNAs or at least cellular machineries to produce them. Evolutionarily, miRNAs seem to be younger than siRNAs. miRNAs function primarily in multicellular organisms, although they have recently been identified in a unicellular alga, *Chlamydomonas reinhardtii*. Certain DNA viruses also express miRNAs. Although 20–30-nucleotide small RNAs have not been identified in archaea and eubacteria (the cells of which lack a nucleus), Argonaute proteins — the effector molecules of small RNAs — are present in some of these organisms.

How are small RNAs made?

Generally, by fragmentation of longer RNA sequences. The precursors of miRNAs and siRNAs are dsRNAs, which are processed to small RNAs by dedicated sets of enzymes and other proteins (Box 2, page 416).

Where do the dsRNAs come from?

Depending on the class of small RNA, the source of precursor dsRNA differs. For siRNAs, dsRNA can form when complementary DNA strands are transcribed into RNA sequences. Viral infection of a cell can also supply dsRNAs, as many viruses form RNAs of both sense and antisense polarity during replication of their genomes, and this can trigger an RNAi response by the cell, as part of its antiviral defence. By contrast, miRNAs are excised from 'purpose-built', genome-encoded RNA precursors that fold into long hairpins resembling dsRNA. The expression of miRNA-encoding genes and those encoding mRNAs are controlled very similarly and involve the same RNA-synthetic machinery, including the enzyme RNA polymerase II.

Box 1 | Prominent members of the RNA family

Classic RNAs mediating protein synthesis

mRNAs (messenger RNAs) Transcripts of protein-coding genes that act as templates for protein synthesis.

rRNAs (ribosomal RNAs) RNA constituents of the ribonucleoprotein particles known as ribosomes, which mediate the decoding of mRNAs to the amino-acid sequences of proteins.

tRNAs (transfer RNAs) Adapter molecules carrying individual amino acids to the site of protein synthesis that recognize specific codons in mRNA.

Non-coding regulatory RNAs

siRNAs (small interfering RNAs) Small RNAs (20–25 nucleotides in length) formed through cleavage of long double-stranded RNA molecules. siRNAs are particularly important for taming the activity of transposons and combating viral infection, but they can also regulate protein-coding genes. Synthetic siRNAs can also be artificially expressed for experimental purposes.

miRNAs (microRNAs) Small RNAs (20–25 nucleotides in length) that are encoded by specific genes and function in repressing mRNA translation or in mRNA degradation in plants and animals. They are processed from long, single-stranded RNA sequences that fold into hairpin structures.

piRNAs (Piwi-associated RNAs) Small RNAs (25–30 nucleotides in length) that are generated from long single-stranded precursors. They function in association with the Piwi subfamily of Argonaute proteins, and are essential for the development of germ cells.

Longer non-coding RNAs RNAs of 70 to thousands of nucleotides that participate in various cellular processes, including mRNA splicing and ribosome biogenesis. **H.G. & W.F.**

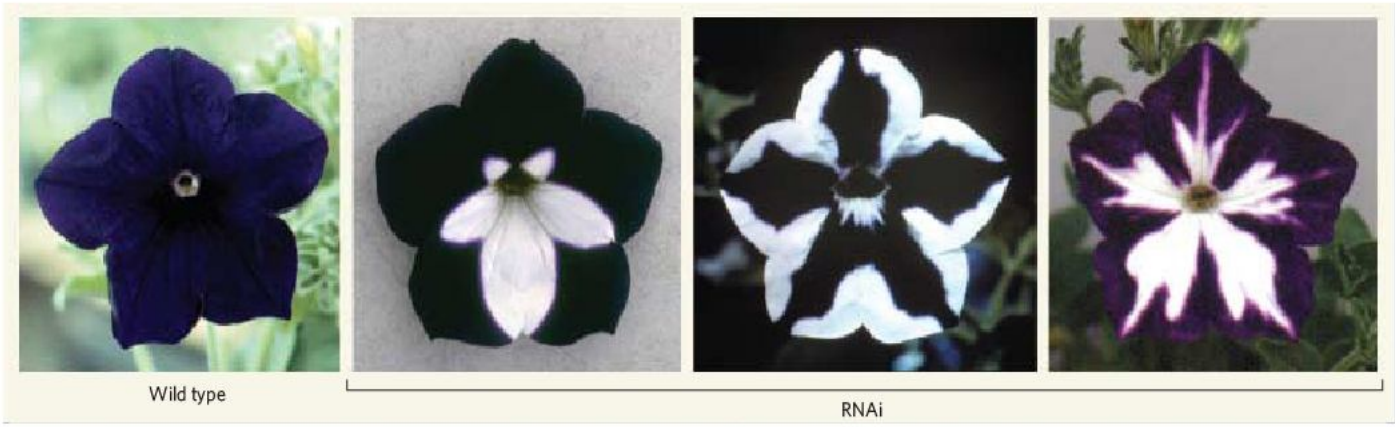


Figure 1 | First phenotypic description of RNA interference. White sections in petunia flowers represent areas where RNAi has silenced a gene involved in flower coloration.

How do small RNAs function?

They recognize their RNA targets by sequence-specific base-pairing. The outcome of the small-RNA–mRNA association depends on the degree of complementarity between the two sequences. When base-pairing is perfect, or almost perfect, as is the case for siRNAs (and possibly piRNAs), the target mRNA is cleaved in the middle of the small-RNA–mRNA duplex. Most plant miRNAs and some animal miRNAs function similarly. But most animal miRNAs base-pair imprecisely with mRNAs to repress their translation or to induce their breakdown. Irrespective of base-pairing precision, small RNAs rely on proteins of the Argonaute family for their activity. In fact, it is the protein partners of small RNAs that bring about repression of translation or mRNA cleavage; small RNAs act only as guides to tell Argonaute proteins which mRNAs to target.

So can their mRNA targets be predicted through sequence analysis?

Unfortunately, the devil is in the detail — at least in the case of animal miRNAs, which mostly base-pair to their mRNA targets with limited complementarity. Although sequence analyses have revealed some criteria for interaction between miRNA and mRNA, and many bioinformatics tools for target identification are available, sequence-based predictions frequently yield false positives or miss true targets. So identification of bona fide miRNA targets requires extensive experimentation. By contrast, most targets of siRNAs and plant miRNAs can be reliably predicted on the basis of near-perfect sequence complementarity.

To prevent protein synthesis, isn't it simpler to stop mRNA production?

Yes. Intuitively, terminating transcription seems a much more obvious mechanism. But a block in protein production always lags behind a block in transcription — even if transcription is stopped, mRNA sequences that have already been made can still be translated into proteins. So by targeting the existing mRNA pool, small RNAs block or attenuate protein synthesis very rapidly and, occasionally, even reversibly. In addition, because individual small RNAs can simultaneously target tens if not hundreds of mRNAs for different proteins, they are well suited to coordinate the expression of genes that function in the same or related pathways. For example, during zebrafish embryonic development, a specific miRNA, *miR-430*, targets hundreds of mRNAs for rapid degradation, facilitating embryos' transition to a new developmental programme that requires a separate set of proteins. The ability of different miRNAs to concurrently target several sequences of the same mRNA further increases their potential to fine-tune gene expression.

Is all of this small-RNA-mediated regulation post-transcriptional?

No — small RNAs also affect DNA transcription, particularly in plants and fission yeast. They do this by sequence-specific targeting of chromatin (complexes of DNA with histone proteins), converting it to the heterochromatin form that is not easily accessible to the transcriptional machinery. Strikingly, in some lower eukaryotes small RNAs also direct massive genomic DNA rearrangements. In mammals, however, there is currently only limited evidence for small-RNA functions other than post-transcriptional regulation.

Do small RNAs always silence gene expression?

In some conditions, small RNAs may also activate gene expression, although the mechanisms are currently not well understood. Indeed, a liver-specific miRNA, *miR-122*, is even needed for successful replication of the hepatitis C virus.

What biological processes do small RNAs regulate?

miRNAs were originally identified in *C. elegans* for their central role in development. Consistent with their function in differentiation and development, expression of many miRNAs is tissue-specific (Fig. 2) or is associated with certain developmental stages. miRNA expression patterns often change in diseases such as cancer. And, as many of the known and predicted miRNA targets have roles in disease, it is widely believed that dysregulation of miRNA expression contributes to disease pathology. It is less clear whether siRNAs have similarly important functions, although in plants they have already been identified as essential players in the regulation of stress resistance. In fission yeast and plants, siRNAs contribute to heterochromatin formation.

And what is the link to viruses?

The use of RNAi as a defence mechanism against viruses may have been a driving force in the evolution of the siRNA pathway. In plants, siRNAs are an essential layer of antiviral defence. Also, in plants and invertebrates, siRNAs silence mobile genetic elements called transposons, which would otherwise 'jump' around the genome and disrupt cellular genes. It is not well known whether these small-RNA functions are also crucial in vertebrates, in which the invention of a protein-based adaptive immune response may have reduced reliance on antiviral RNAi activity.

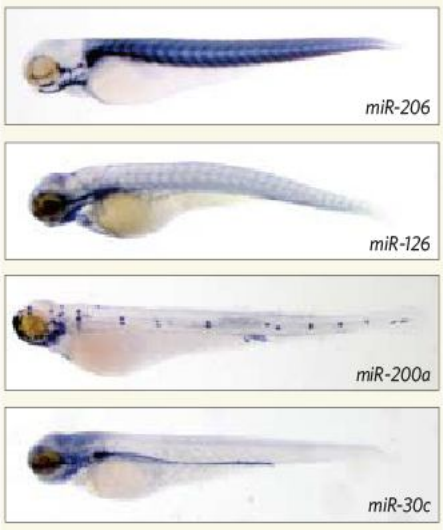
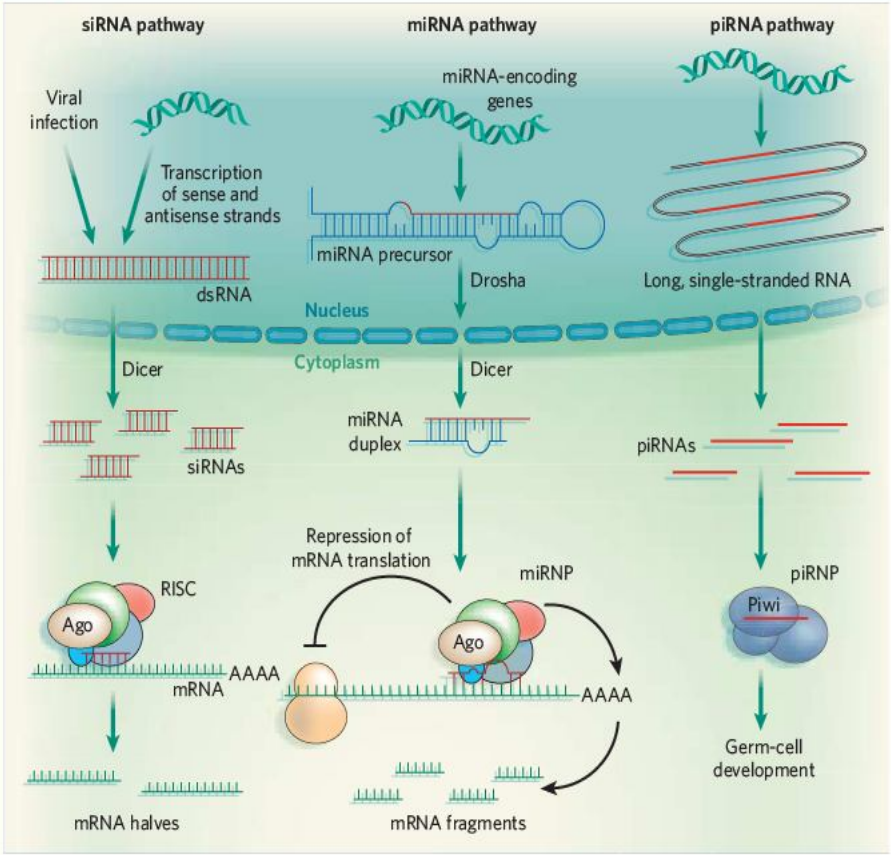


Figure 2 | miRNAs have tissue-specific functions. In zebrafish embryos, localization patterns of individual miRNAs indicate that their activity could be limited to tissues and organs in which they are expressed. As indicated by blue staining, *miR-206* is mainly expressed in the muscle, *miR-126* in the blood vessels and the heart, *miR-200a* in the lateral-line system (a mechanosensory system detecting water motion) and sensory organs, and *miR-30c* in the kidney precursor.

Box 2 | Formation and function of small RNAs



Small RNAs are generally produced by fragmentation of longer precursors. **Small interfering RNAs (siRNAs)** are processed from double-stranded RNAs (dsRNAs) that form by base-pairing of complementary RNAs. An enzyme called Dicer cleaves dsRNA into shorter double-stranded siRNAs that are roughly 20 base pairs long. One siRNA strand then assembles into an effector complex known as an RNA-induced silencing complex (RISC). This complex uses the siRNA guide to identify mRNAs with a sequence perfectly complementary to the siRNA. RISC then cleaves the mRNA in the middle of the mRNA–siRNA duplex, and the resulting mRNA halves are degraded by other cellular enzymes. Under some circumstances, siRNAs might associate into complexes other than RISC (not shown) that function in the nucleus and silence gene transcription. **MicroRNAs (miRNAs)** are processed from specific genome-encoded precursors, which fold into intramolecular hairpins containing imperfectly base-paired segments. The processing generally occurs in two steps, and is catalysed by the enzymes Drosha (in the nucleus) and Dicer (in the cytoplasm). One strand of the resulting miRNA duplex, resembling an siRNA, then incorporates into a RISC-like miRNA–ribonucleoprotein (miRNP) complex. The main components of RISC and miRNPs are proteins of the Argonaute (Ago) family.

Depending on the level of complementarity, miRNAs induce mRNA degradation or repress their translation. Unlike the siRNA pathway, miRNA-mediated degradation is initiated by enzymatic removal of the mRNA poly(A) tail. **Piwi-associated RNAs (piRNAs)** are generated from long, single-stranded precursors in a process independent of Drosha and Dicer. These small RNAs associate with a subfamily of Argonaute proteins called Piwi proteins. Tens of thousands of piRNAs have been identified, although they are far from understood. It is, however, known that, together with their Piwi partners, they are essential for the development of germ cells. **H.G. & W.F.**

Does our collection of small RNAs set us apart from other species?

Some miRNAs are highly conserved, but others vary greatly among organisms; some differ even among primates, for example between apes and humans. With the emerging view that regulation of protein activity could be as vital to evolution as fidelity of the protein sequence itself, it is tempting to speculate that miRNAs influence evolution. Relatively simple requirements for miRNA–mRNA interaction facilitate the development of new regulatory relationships between these sequences, possibly contributing to the evolution of new functions. Perhaps, therefore, it is not surprising that a large fraction of tissue-specific miRNAs operates in the brain.

Are small RNAs restricted to the cells in which they are made?

In *C. elegans* and plants, dsRNAs or siRNAs can move between cells or even longer distances. For example, siRNAs spread through the vascular system of plants, which possibly aids their function in antiviral immunity. And in *C. elegans*, an efficient system of siRNA amplification ensures the maintenance of gene silencing even after the initial 'trigger siRNA' is gone, allowing siRNA to spread to the organism's progeny. A similar amplification system does not occur in mammals. As for miRNAs, their very specific localization patterns (Fig. 2), and the absence of developmental changes in *C. elegans* mutants of the dsRNA transport machinery, suggests that miRNAs are stationary.

Will small RNAs be useful as therapeutic agents or targets?

This is a hot topic of research. siRNAs have the potential to silence disease-relevant genes that cannot be shut down with available drugs. Moreover, the so-called oncomiRs — miRNAs that promote cancer — may themselves be targets for shut-down. But we are still a long way from translating activity observed in a defined experimental system into an effective therapeutic drug. One of the most problematic issues is how to get small RNAs efficiently and specifically to their target site of action in the human body. But regardless of their therapeutic potential, siRNAs have already revolutionized basic biomedical research. The use of synthetic siRNAs, or their short hairpin RNA or dsRNA precursors, allows researchers to repress the function of a gene of interest or even to perform genome-wide RNAi screens to unravel entire biological pathways with unprecedented ease and speed.

So what of the future?

New classes of small RNAs continue to be discovered, and it is unlikely that we have found them all. Even for the known classes, we often have only a very limited understanding of what they do and how they do it. Identification of miRNA targets is another challenge, as is identifying other, currently hypothetical, modes of small-RNA action. Owing to their base-pairing potential, small RNAs could modify local mRNA structures, allowing for alternative mRNA splicing and modulating interactions of mRNAs with proteins. So watch this space. ■

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REVIEWS

Catalytic C–H functionalization by metal carbenoid and nitrenoid insertion

Huw M. L. Davies[1] & James R. Manning[1]

Novel reactions that can selectively functionalize carbon–hydrogen bonds are of intense interest to the chemical community because they offer new strategic approaches for synthesis. A very promising 'carbon–hydrogen functionalization' method involves the insertion of metal carbenes and nitrenes into C–H bonds. This area has experienced considerable growth in the past decade, particularly in the area of enantioselective intermolecular reactions. Here we discuss several facets of these kinds of C–H functionalization reactions and provide a perspective on how this methodology has affected the synthesis of complex natural products and potential pharmaceutical agents.

In 2006, 31 new chemical entities were introduced to the world pharmaceutical market and 2,075 molecules were in phase I or II of clinical development[1]. The majority of these were small-molecule (relative molecular mass <1,000) organic compounds[2]. As knowledge about the specific interactions of drugs *in vivo* increases, often so does the structural complexity of new drug targets. A major obstacle to the development of such drugs is the difficulty associated with synthesizing large quantities in an economical fashion, because complex multi-step syntheses are usually required. In the general media, it is often overlooked that the accessibility of the components required for these new treatments will often govern their eventual success or failure. Likewise, a design element of any pharmaceutical agent is the expectation that the target compounds can be made economically. Therefore, new strategies for synthesis can become enabling technologies, making available new targets and materials that would have been previously out of range. For example, new methodologies such as metal-catalysed cross-coupling[3] and olefin metathesis[4–6] have rapidly become central transformations in the synthesis of new pharmaceutical agents. Selective C–H functionalization is a class of reactions that could lead to a paradigm shift in organic synthesis, relying on selective modification of ubiquitous C–H bonds of organic compounds instead of the standard approach of conducting transformations on pre-existing functional groups. The reactive sites in each type of transformation are very different, as illustrated in Fig. 1.

The many opportunities associated with C–H functionalization has made this field an active area of research. Organometallic chemists have focused much attention on developing 'C–H activation' strategies, whereby a highly reactive metal complex inserts into a C–H bond, activating the system for subsequent transformations[7–9]. One of the major challenges associated with this chemistry has been to render it catalytic in the metal complex[10]. A partial solution to this problem has been to use neighbouring functionality to direct less reactive metal complexes to the site for functionalization. Numerous reviews have been written about this method for C–H functionalization[11–17]. Here, however, we highlight another approach, in which a divalent carbon (carbene)[18] or a monovalent nitrogen (nitrene)[19], coordinated to a metal complex, inserts into a C–H bond[20]. This alternative approach offers many advantages over the metal-induced C–H insertion because the reactions exhibit high turnover numbers and can lead to high levels of selectivity, both in terms of regioselectivity and stereoselectivity (Fig. 2).

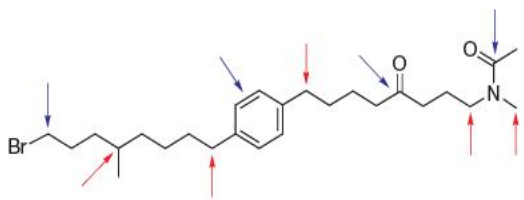

Figure 1 | Synthesis by functional group modification compared to C–H functionalization. Traditional sites for modification of organic molecules (indicated by blue arrows) rely on reactive (polarizable) functional groups. Such modes of reactivity include oxidation/reduction, aromatic substitution, and nucleophilic/electrophilic attack. Sites for direct functionalization of C–H bonds (red arrows) often have adjacent 'activating' groups, but can also occur at isolated positions.

C–H functionalization by metal carbenoids

The standard method for generating the transient metal carbenes is by metal-induced extrusion of nitrogen from diazo compounds[21].

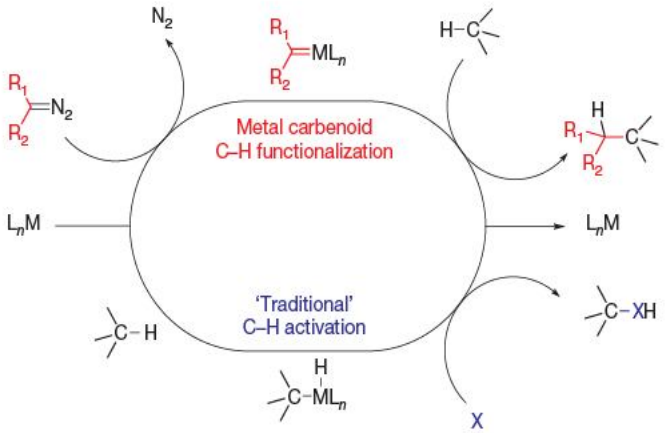

Figure 2 | Metal carbenoid C–H functionalization versus the 'traditional' C–H activation. In a traditional C–H activation manifold, the highly reactive metal complex (M = metal, L = ligand) inserts into a C–H bond. Regeneration of the active metal complex to form the C–H activation product has proved difficult. In contrast, C–H functionalization via a metal carbenoid approach typically uses a high-energy diazo compound and loss of nitrogen provides the driving force for the energetically unfavourable formation of the carbenoid. The highly reactive carbenoid species then inserts into a C–H bond to form the C–H activation product and liberates the metal catalyst for another cycle.

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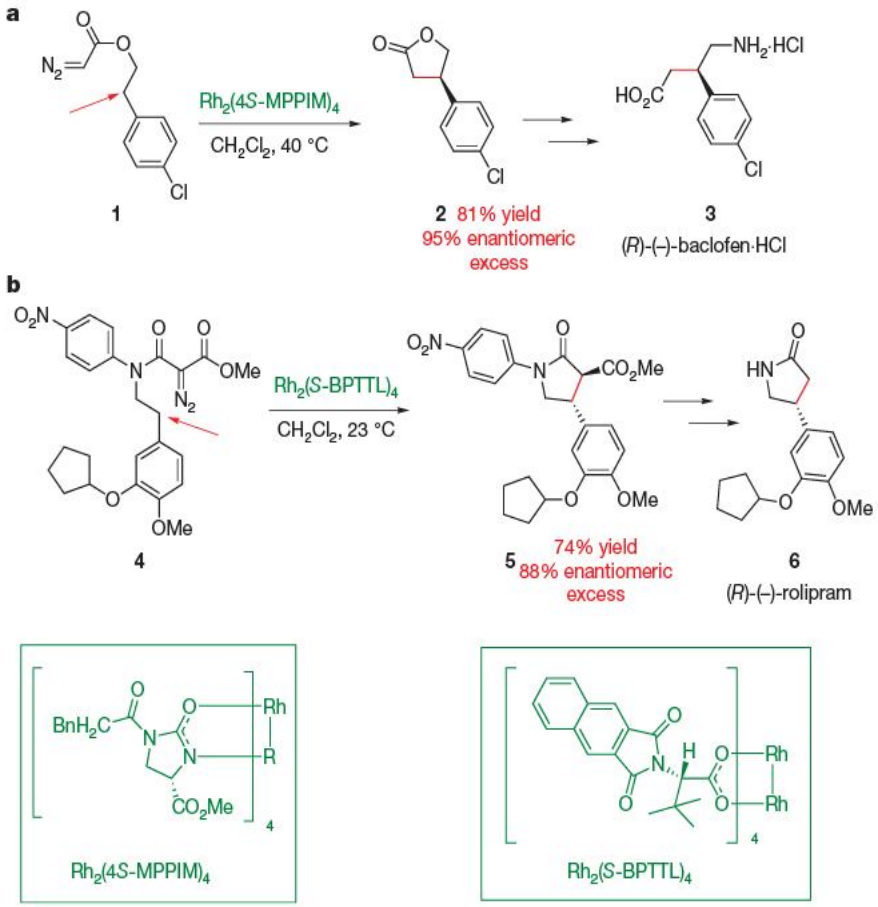

Figure 3 | Intramolecular C–H insertions. a, The intramolecular C–H insertion of an acceptor substituted carbenoid catalysed by the chiral rhodium carboxamidate catalyst Rh_2(4S-MPPIM)$_4$ is the key step in an enantioselective synthesis of the $GABA_B$ receptor agonist (*R*)-(–)-baclofen (ref. 24). **b**, The intramolecular C–H insertion of an acceptor/acceptor-substituted carbenoid catalysed by the chiral rhodium carboxylate catalyst Rh_2(*S*-BPTTL)$_4$ results in a concise synthesis of the phosphodiesterase type IV inhibitor (*R*)-(−)-rolipram (ref. 25).

The challenges of regioselectivity associated with the carbene-induced C–H functionalization meant that most of the early advances in this field were achieved in systems capable of intramolecular reactions[21–23]. Because the rhodium carbenoid and the reacting C–H bond are connected by a suitable tether, they are brought into close proximity, leading to a favourable regioselective transformation. By using a chiral catalyst, the C–H insertion can be made enantioselective, favouring the formation of one mirror image of the product over the other. This type of approach has been used in the synthesis of various pharmaceutical agents, such as (*R*)-(−)-baclofen[24] (**3**) and (*R*)-(−)-rolipram[25] (**6**) (Fig. 3).

In terms of strategic reactions, controllable intermolecular transformations would be much more powerful because the sequence of steps required to make the substrate for an intramolecular reaction would no longer be needed. Controlling the regioselectivity of intermolecular C–H insertions, however, has been challenging, particularly in the case of the most commonly used metal carbenoids derived from acceptor-only substituted diazoacetates[22,26]. The metal carbenoids behave as very electrophilic species and the electron-withdrawing ester group reinforces the high reactivity, generating a system characterized by poor regioselectivity between different C–H bonds[27–29]. In recent years, extensive efforts have been made to attenuate the carbenoid reactivity by altering the nature of the catalysts, with some improvements having been made by using very bulky ligands with copper[30] and silver[31] complexes. The major breakthrough in this field, however, was the discovery that carbenoids functionalized with both donor and acceptor groups were much more chemoselective than the traditional carbenoids, as shown in Fig. 4[18,32,33].

The synthetic potential of the donor/acceptor carbenoids is illustrated in a direct enantioselective synthesis of the most active enantiomer of *threo*-methylphenidate (Ritalin) (compound **9**).

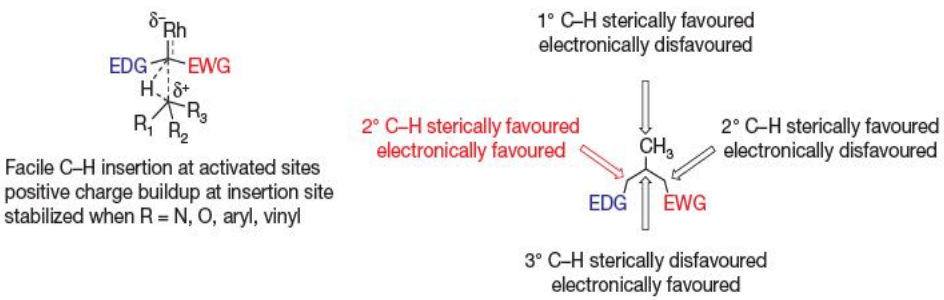

Figure 4 | Controlling factors of carbenoid reactivity. The substituents attached to the carbenoid help to modulate its reactivity. The presence of both an electron-donating group (EDG) and an electron-withdrawing group (EWG) is necessary to reduce carbene dimerization pathways and increase selectivity for intermolecular reactions. During the C–H activation event, a partial positive charge build-up occurs at the carbon undergoing C–H functionalization. Sites adjacent to functionality that can stabilize this polarization are considered to be electronically 'activated' towards carbenoid reactions (1°, 2° and 3° represent primary, secondary or tertiary sites, indicating the number of substituents at a particular carbon site).

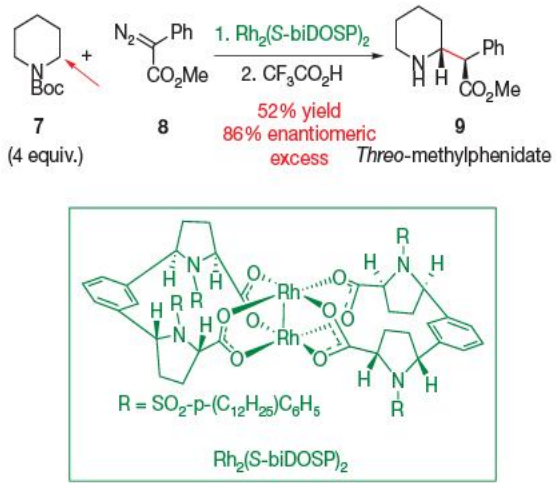

Figure 5 | Ritalin synthesis. A concise, stereoselective synthesis of the biologically active enantiomer of the pharmaceutical agent Ritalin (*threo*-methylphenidate) was achieved using the bridged, chiral rhodium catalyst $Rh_2(S\text{-biDOSP})_2$ (ref. 38). Boc, *tert*-butoxycarbonyl.

Ritalin is an important drug for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and, as an old drug, is sold as a racemate. The most active enantiomer of Ritalin has been marketed as an independent therapeutic agent[34]. Its synthesis using conventional functional group manipulations requires multiple steps[35,36]. The C–H functionalization approach to Ritalin, conversely, is direct[37,38]. The $Rh_2(S\text{-biDOSP})_2$[39] catalysed reaction of *N*-protected piperidine (compound **7**) with methyl phenyldiazoacetate (compound **8**) followed by removal of the protecting group leads to the rapid synthesis of (*R*,*R*′)-(+)-methylphenidate (compound **9**) in 86% enantiomeric excess (Fig. 5)[38].

C–H functionalization can provide complementary approaches to achieve transformations equivalent to some of the classic reactions of organic synthesis[40–45]. For example, C–H functionalization adjacent to oxygen can lead to products that would be commonly derived from an aldol reaction (Fig. 6)[44,46]. In the specific example used to illustrate this concept, other key elements that control the selectivity of this chemistry are demonstrated. The carbenoid has an electronic preference to functionalize C–H bonds in which the carbon can stabilize positive charge build-up because the C–H insertion has the partial characteristic of a hydride abstraction event[47,48]. In this case, selective functionalization occurs adjacent to the siloxy group rather than the more electron-withdrawing acetoxy group in compound **10**. Additionally, if there is good steric differentiation at the C−H insertion site, high levels of diastereoselectivity can be achieved. Thus, compound **11** is formed in 92% yield, >94% diastereomeric excess and 72% enantiomeric excess[44].

C–H functionalization adjacent to nitrogen can lead to products that would be typically formed from a Mannich reaction, as illustrated in the direct synthesis of β-amino ester **13** (ref. 42). This example illustrates the important controlling influences in steric factors, because the electronically most activated site in compound **12**, the benzylic carbon, is sterically inaccessible and selective functionalization occurs at the *N*-methyl group[42,49,50].

A further advantage of the carbenoid-induced C–H functionalization is the possibility of the C−H insertion step initiating a cascade sequence. A spectacular example of such an event is termed the 'combined C–H activation/Cope rearrangement'[51–56]. One of the earliest examples of this reaction is shown between the vinyldiazoacetate **15** and 1,3-cyclohexadiene in Fig. 7. Before the C–H functionalization is complete, a rearrangement occurs to form a 1,4-cyclohexadiene derivative **16** with exceptional enantiocontrol. The entire process is believed to occur via a concerted, ordered transition state that leads to higher stereoselectivity than is normally observed in a direct C–H insertion. This transformation has been used in a very concise enantioselective formal synthesis of the antidepressant sertraline (Zoloft, Fig. 7)[51].

An even more elaborate sequence of events has been developed for the enantioselective synthesis of 4-substituted indoles. Indoles are present in a number of pharmaceutical agents[57] and there has been much interest in the enantioselective synthesis of 1-aryl-1-indolylalkyl derivatives. Normally the indole is functionalized at the 2- or 3-position because these are the most reactive sites using conventional chemistry[58–60]. In contrast, the C–H functionalization strategy results in a very efficient method for generating 4-substituted indoles with high enantioselectivity (Fig. 8)[56]. These types of

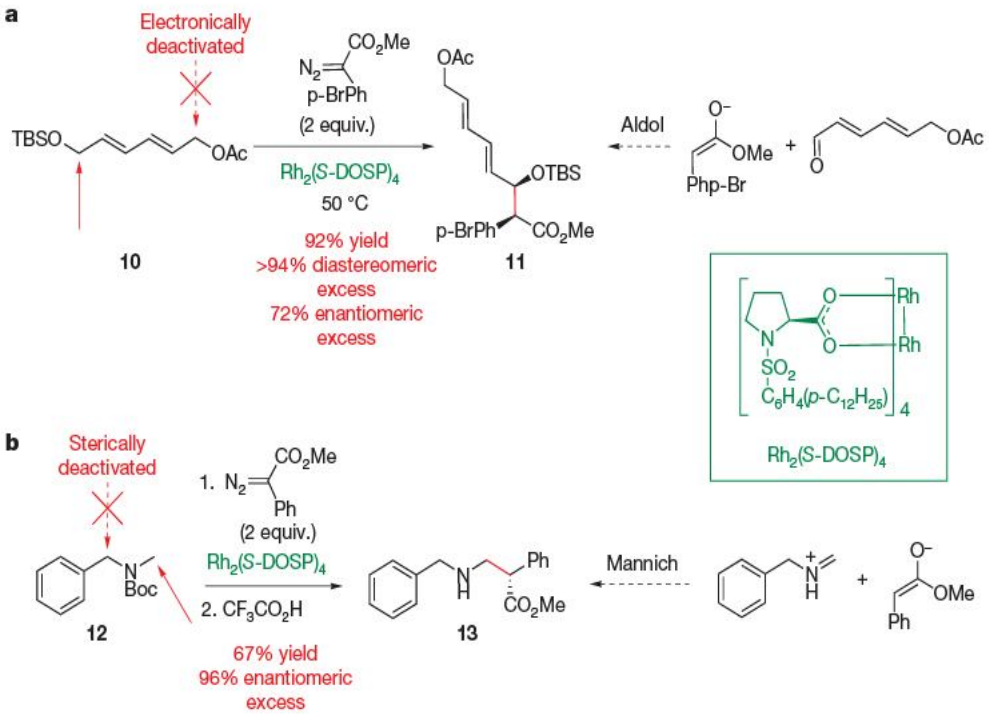

Figure 6 | C–H functionalization as a strategic reaction. a, C–H insertion α to oxygen generates products that would classically be formed via an aldol reaction. The insertion occurs preferentially at the site adjacent to the more electron-rich siloxy-protected oxygen (ref. 44) **b**, C–H insertion α to nitrogen generates the products formally derived from a Mannich reaction, with high levels of enantiocontrol (ref. 42). TBSO, *tert*-butyldimethylsiloxy.

Figure 7 | Combined C–H activation/Cope rearrangement. The C–H functionalization with a vinyldiazoacetate begins at the allylic C–H bond, but is interrupted when a Cope rearrangement occurs (see blue intermediate) to give the combined C–H activation/Cope rearrangement product with exceptionally high enantioselectivity (ref. 51).

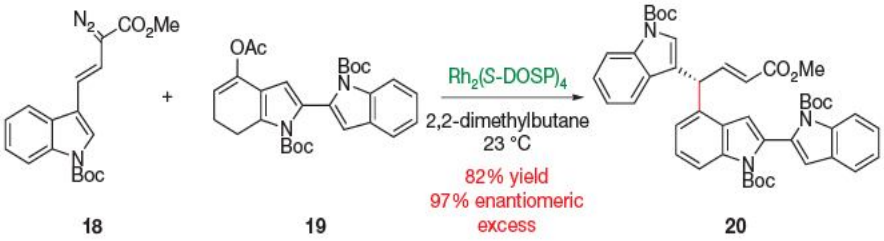

Figure 8 | *Tris*-indole synthesis. The product of the combined C–H activation/Cope rearrangement between the vinyldiazoacetate **18** and the 4-acetoxy-6,7-dihydroindole **19** undergoes loss of acetic acid to generate the aromatized *tris*-indole compound **20** in good yield and in high enantiomeric excess (ref. 56).

compounds and related structures have not been extensively studied as potential therapeutic agents, presumably because they were not readily accessible.

A powerful enabling synthetic methodology should greatly simplify the total synthesis of complex natural products. Notable examples of this have been illustrated in the synthesis of the natural products derived from the West Indian gorgonian coral *Pseudopterogorgia elisabethae* (Fig. 9)[61]. Previous syntheses have struggled with controlling the stereochemistry at the three stereocentres indicated in red, primarily because the natural products lack

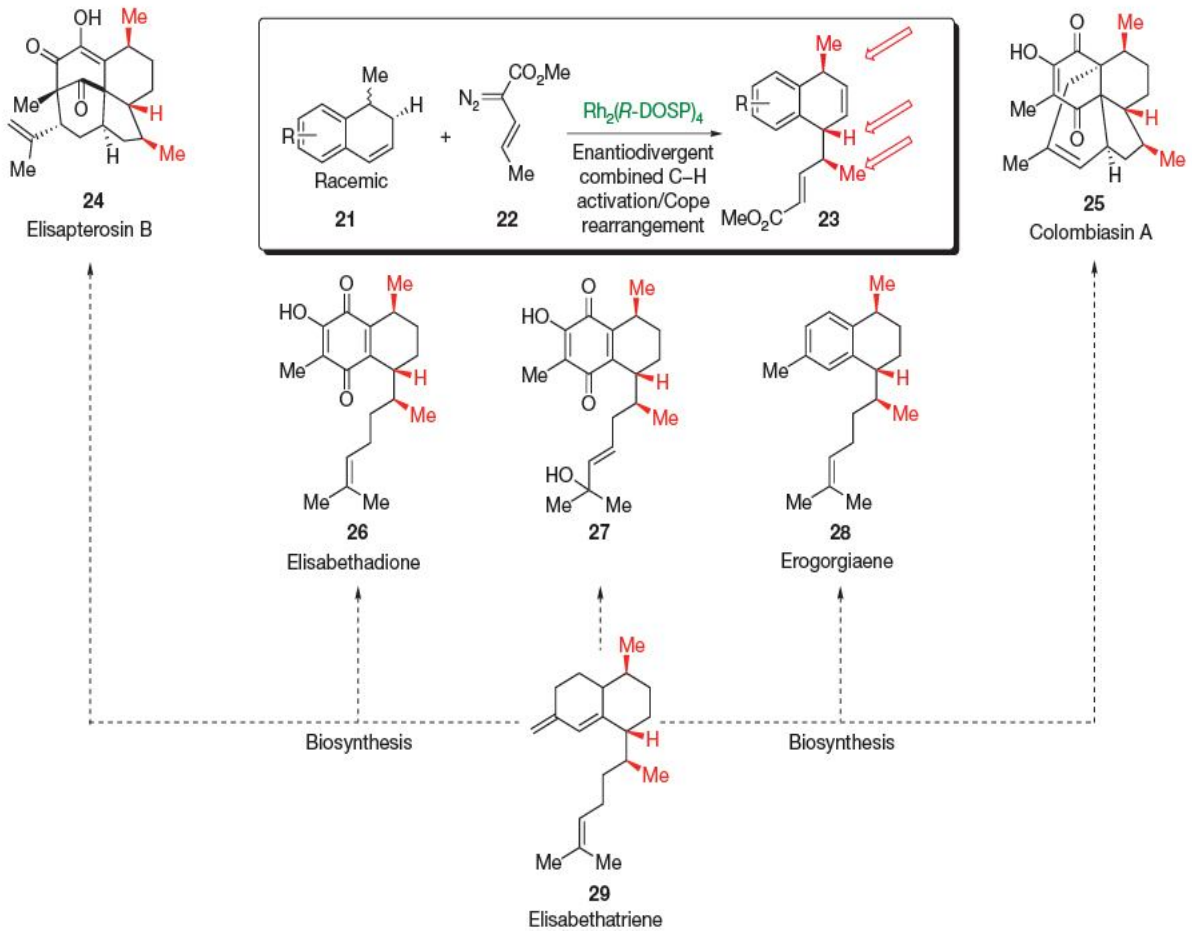

Figure 9 | Application of C–H functionalization to natural product synthesis. A series of diterpene natural products have been isolated from the gorgonian coral *Pseudopterogorgia elisabethae*. All are derived biosynthetically from (+)-elisabethatriene and share the same configuration at the three stereocentres shown by red arrows (ref. 61). One of the most challenging aspects in synthesizing these molecules is controlling the stereochemistry at these sites because of the lack of neighbouring functional groups. A powerful feature of the combined C–H activation/Cope rearrangement is the ability of the chiral catalyst Rh_2(*R*-DOSP)$_4$ to differentiate between the enantiomers of a racemic substrate to generate all three stereocentres in a single step.

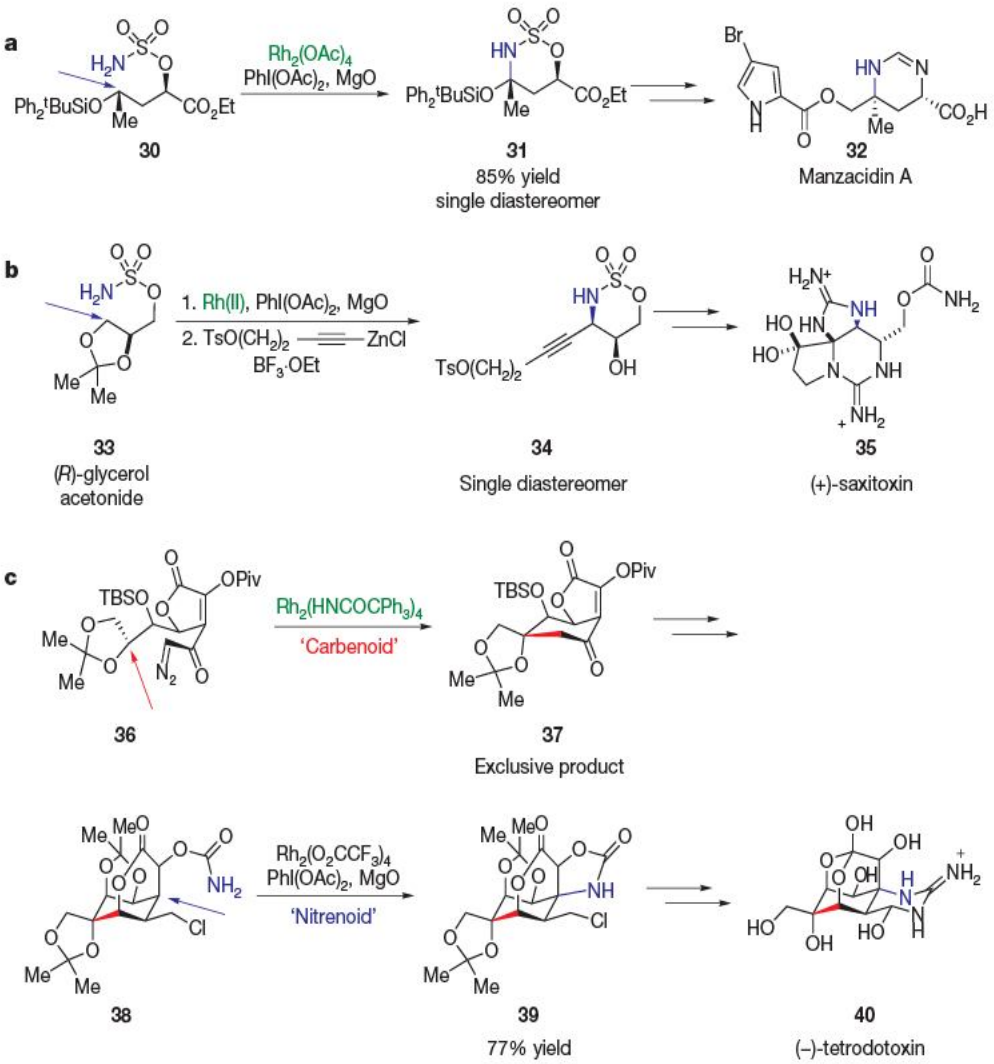

Figure 10 | Application of C–H amination to natural product synthesis. **a**, **b**, Sulphamate esters provide excellent precursors to nitrenes that can undergo highly diastereoselective intramolecular insertions into tertiary C–H bonds. Du Bois has shown the potential of this methodology with syntheses of the marine alkaloid manzacidin A (ref. 74) and the natural product (+)-saxitoxin, an ion channel blocker (ref. 75). **c**, An elegant synthesis of the potent marine poison (±)-tetrodotoxin by Du Bois and colleagues illustrates the utility and complementarity of C–H functionalization by metal carbenoids and nitrenoids (ref. 76). $Ph_2{}^tBuSiO$, diphenyl-*tert*-butylsiloxy; Piv, pivalate.

suitable functional groups as handles in the synthetic transformations[62–67]. In contrast, an enantiodivergent C–H functionalization of racemic dihydronaphthalenes **21** generates the core structures **23** with high stereoselectivity at all three stereocentres. In the presence of the chiral catalyst Rh_2(*R*-DOSP)$_4$ (ref. 68), the other enantiomer of the dihydronaphthalene **21** reacts in an entirely different manner to form cyclopropanation products[69]. This reaction can be conducted with a variety of dihydronaphthalene derivatives and has been applied to very efficient syntheses of a range of natural products including elisapterosin B[70] (compound **24**), colombiasin A[70] (compound **25**), elisabethadione[71] (compound **26**), the *p*-benzoquinone **27**[71] and erogorgiaene (compound **28**)[69].

C–H functionalization by metal nitrenoids

In recent years, metal nitrenoid complexes have also been shown to be capable reagents for C–H functionalization, a reaction that is usually called C–H amination[14,72,73]. Some spectacular intramolecular examples have been reported by Du Bois and colleagues for the synthesis of complex natural products such as manzacidin A[74] (compound **32**), (+)-saxitoxin[75] (compound **35**) and (−)-tetrodotoxin[76] (compound **40**) (Fig. 10). These transformations illustrate how, by judicious choice of substrates, the C–H amination can be conducted in the presence of a range of functional groups. Generally, the most broadly used precursors to the transient metal nitrenes have been aryliminoiodinanes, which are decomposed by a suitable metal complex[77]. In the syntheses described here, the aryliminoiodinanes are produced *in situ* from the corresponding amine, which makes the overall transformations even more attractive[78,79].

Lebel and colleagues have shown that *N*-tosyloxyamides such as compound **41** are also efficient precursors to transient metal

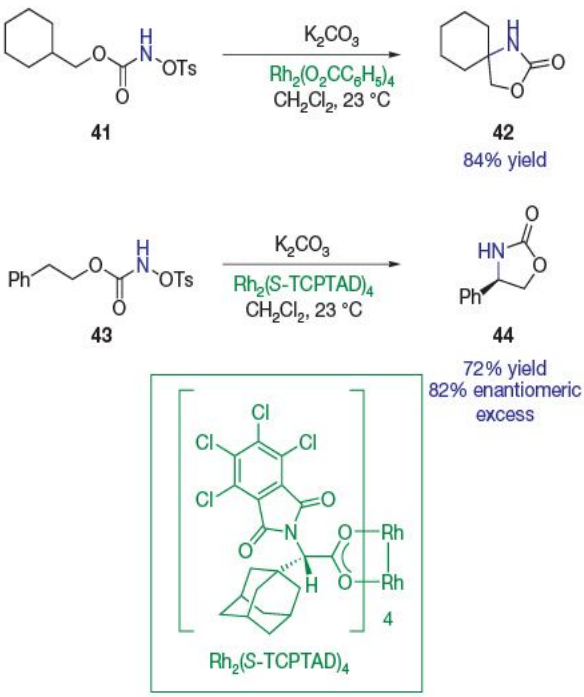

Figure 11 | Enantioselective C–H amination. Lebel and colleagues have developed an alternative method for the *in situ* generation of metal nitrenoids using *N*-tosyloxycarbamates as the precursors. This method avoids the generation of a stoichiometric amount of iodobenzene, a drawback to the use of hypervalent iodine reagents that are commonly used to generate nitrene precursors *in situ*. Lebel's method proceeds with high efficiency (ref. 80), and when the chiral catalyst Rh_2(*S*-TCPTAD)$_4$ is used, good levels of enantioselectivity can be achieved (ref. 82).

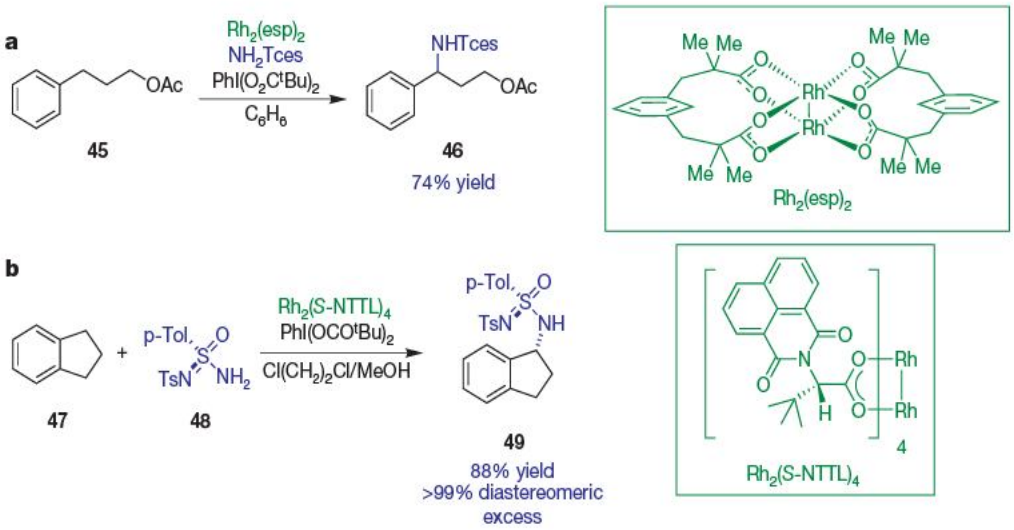

Figure 12 | *In situ* generation of metal nitrenoid precursor. a, The bridged, achiral rhodium catalyst $Rh_2(esp)_2$, developed by Du Bois and co-workers specifically for nitrenoid reactions, has demonstrated remarkable efficiency in both intra- and intermolecular reactions with catalyst loadings as low as 2 mol.% (ref. 84). **b**, Exceptionally high diastereoselectivity can be achieved in intermolecular C–H amination reactions when a 'matched' reaction is carried out using a chiral sulphonamide as the nitrene precursor and a chiral rhodium catalyst (ref. 87). 'esp' is a trivial name for the catalyst (structure shown). Tces, 2,2,2-trichloroethoxysulphonyl.

nitrenoids[80,81]. By conducting this nitrene insertion with chiral dirhodium catalyst $Rh_2(S\text{-TCPTAD})_4$, enantioselective transformations can be achieved, as illustrated in Fig. 11[82]. The site for C–H amination is governed by the length of the tether and in general five-membered rings are preferred.

Du Bois and colleagues have developed a bridged achiral rhodium catalyst $Rh_2(esp)_2$ that has performed well at low catalyst loadings in both intra- and intermolecular C−H amination reactions (Fig. 12)[83–85]. Enantioselective intermolecular reactions of metal nitrenes are less developed than the parallel reactions of metal carbenes, although some significant examples have been reported[81,82,86,87]. A conceptually interesting approach to stereoselective intermolecular nitrene chemistry involves the use of a chiral sulphonamide (compound **48**) as the amine source in the reactions. Exceptionally high diastereoselectivity can be obtained in matched reactions between the appropriate enantiomers of a chiral sulphonamide and a chiral rhodium catalyst[87]. Given the ubiquity of nitrogen atoms in biologically active compounds, C−H amination as an enabling technology in pharmaceutical synthesis has broad potential. A number of efficient transformations have been achieved at benzylic positions, but broad application of this chemistry is still relatively limited.

Future directions

C–H functionalizations by means of metal carbenoids and metal nitrenoids are rapidly becoming very general strategic reactions for the synthesis of natural products and pharmaceutical targets. The intramolecular C−H insertion of metal carbenoids is now a well-established transformation, while the corresponding intramolecular C−H amination has, in the last few years, been shown to be applicable to the synthesis of highly complex natural product targets. The recognition that donor/acceptor functionalized carbenoids are highly selective intermediates has opened up enantioselective intermolecular C–H insertion as a powerful transformation. Enantioselective intermolecular C−H amination is also developing well and is likely to see broad synthetic application. During the next few years this field is likely to undergo rapid expansion as improved chiral catalysts and even more selective reagents are developed. It has already been shown that these reactions can be considered as complementary to some of the classic reactions of organic synthesis and this will be further emphasized as additional ingenious applications of this chemistry to total synthesis are described. As the synthetic uses of C–H functionalization become more fully appreciated, its application as an enabling technology for drug discovery and synthesis will become a common practice.

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ARTICLES

Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu

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Human cells possess an antiviral activity that inhibits the release of retrovirus particles, and other enveloped virus particles, and is antagonized by the HIV-1 accessory protein, Vpu. This antiviral activity can be constitutively expressed or induced by interferon-α, and it consists of protein-based tethers, which we term 'tetherins', that cause retention of fully formed virions on infected cell surfaces. Using deductive constraints and gene expression analyses, we identify CD317 (also called BST2 or HM1.24), a membrane protein of previously unknown function, as a tetherin. Specifically, CD317 expression correlated with, and induced, a requirement for Vpu during HIV-1 and murine leukaemia virus particle release. Furthermore, in cells where HIV-1 virion release requires Vpu expression, depletion of CD317 abolished this requirement. CD317 caused retention of virions on cell surfaces and, after endocytosis, in CD317-positive compartments. Vpu co-localized with CD317 and inhibited these effects. Inhibition of Vpu function and consequent mobilization of tetherin's antiviral activity is a potential therapeutic strategy in HIV/AIDS.

Some human cells possess a poorly defined antiviral activity, the existence of which is indicated by studies of the HIV-1 accessory protein, Vpu, that inhibits the release of certain enveloped virus particles. Specifically, Vpu is required for efficient HIV-1 particle release in certain human cells[1–3], and HeLa cells are a prototypic example of cells that exhibit this requirement[4]. However, Vpu is completely dispensable for efficient HIV-1 particle release in some other human cells and in the African green monkey cell line COS-7[5,6]. Notably, fusion of HeLa cells with COS-7 cells results in heterokaryons that exhibit the properties of HeLa cells[7], suggesting the existence of an inhibitor of virion release that is expressed in HeLa cells, but not in COS cells, and is antagonized by Vpu. Moreover, Vpu stimulates the release of retrovirus particles as diverse as HIV-1 and murine leukaemia virus (MLV) from HeLa cells[4], as well as virus-like particles based on the structural protein of Ebola virus[8], further suggesting that the inhibitor works in a nonspecific way to block envelope virus particle release.

Early analyses of cells infected with Vpu-deleted HIV-1 strains revealed that virions accumulate within intracellular vacuoles and at the cell surface, at the expense of particle release[3,4]. Recent work has shown that the intracellular virions are primarily localized in $CD63^+$ endosomes[6,8], and that they accumulate at this location as a result of their internalization from the cell surface[6,8–10]. Moreover, HIV-1 particles that are retained by cells owing to the absence of Vpu are fully formed and mature, and can be released from cell surfaces by protease, particularly when their endocytosis is blocked[6,8]. Thus, the inhibitor that is counteracted by Vpu seems to induce adherence of nascent virions to the surface of cells, after completion of their assembly, and from where they may be internalized. We recently found that a requirement for Vpu during HIV-1 virion and Ebola-virus-like particle release can be induced or enhanced in fibroblasts or T cells by treatment with interferon-α (IFN-α)[8]. Notably, the nature of the HIV-1 release defect that accompanies Vpu deletion in these IFN-α-treated cells precisely recapitulates that observed constitutively in HeLa cells[6,8]. Thus, these data suggest that the inhibitor that is antagonized by Vpu is an IFN-α-induced, cell-surface-protein-based tether, that we term 'tetherin'. However, although evidence for the existence of tetherin is quite considerable, it is indirect, and the identity of tetherin is unknown.

Identification of tetherin candidates

To identify tetherin candidates we applied several constraints based on the aforementioned studies that predicted its distribution and nature. First, tetherin should be constitutively expressed in a cell line (HeLa) where HIV-1 Vpu is constitutively required for efficient retrovirus particle release[4], but not in cells where Vpu is dispensable (for example, HOS, 293T or HT1080 cells[6,8]; Supplementary Table 1). Second, because a requirement for Vpu during HIV-1 release becomes evident after IFN-α treatment in 293T and HT1080[8] cells (Supplementary Table 1) and is enhanced by IFN-α treatment in Jurkat cells and primary lymphocytes[8], then tetherin expression should reflect this property. Moreover, because Vpu-defective HIV-1 release seems to be blocked after the fission of cell and virion membranes[3,4,6], tetherin should be a secreted or membrane-associated protein. Although none of the aforementioned criteria alone would provide sufficient discriminatory power to identify tetherin, we reasoned that their simultaneous application might reduce candidates to a reasonable number to be tested individually.

Microarray analyses of messenger RNAs expressed in the aforementioned untreated and IFN-α-treated cell lines revealed few (<10) candidates that conformed to the above criteria. Among these was CD317, a membrane protein of unknown function[11], as well as three 'interferon-induced transmembrane proteins', IFITM1, IFITM2 and IFITM3 (Fig. 1a and Supplementary Table 2). On the basis of their expression levels in the various cell lines, CD317 appeared to be the most compelling of these candidates (Fig. 1a and Supplementary Table 2). Indeed, its expression, as measured by microarray or quantitative reverse transcription–polymerase chain reaction (qRT–PCR) analysis, was >20-fold higher in HeLa than in HOS cells, was induced >20-fold by IFN-α in 293T and HT1080 cells, and was induced two- to fivefold in Jurkat cells (Fig. 1b, c). Moreover, IFN-α treatment accentuates the requirement for Vpu in HIV-1 replication in primary lymphocytes[8] and, concordantly, *CD317* mRNA was

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induced by IFN-α in $CD4^+$ enriched lymphocytes from two donors (Fig. 1d).

Transfection of 293T cells with wild-type (HIV-1(WT)) and Vpu-deleted (HIV-1(delVpu)) proviral plasmids generated, as expected, equivalent yields of HIV-1 particles, determined using western blot or infectious virion measurements (Fig. 1e, f). However, HIV-1 (delVpu), but not HIV-1(WT), virion production was strongly inhibited by co-expressed CD317. Notably, Gag protein expression or processing was not affected by CD317 (Fig. 1e), suggesting that the candidate tetherin indeed inhibited particle release. Conversely, similar transient transfection assays revealed that overexpression of IFITM1, IFITM2 or IFITM3 did not affect HIV-1(delVpu) release (data not shown).

Inhibition of retrovirus release by tetherin

Because Vpu affects CD4 expression as well as virion release[12] and because CD4 can interfere with virus release and infectivity[13,14], the effect of the candidate tetherin on HIV-1(WT) and HIV-1(delVpu) replication was analysed using single-cycle HIV-1 replication assays in $CD4^-$ cells. These assays were done in cells that do (HeLa) or do not (293T, HT1080) constitutively express CD317. Furthermore, derivatives of 293T and HT1080 cells that stably expressed CD317 were established via retroviral vector transduction, and qRT–PCR analyses revealed that *CD317* mRNA was only moderately over-expressed (~2–6-fold more abundant than in HeLa cells) in these cell lines. Crucially, stable CD317 expression reduced Vpu-defective HIV-1 virion yield by >100-fold (293T cells) and ~80-fold (HT1080 cells) over a single replication cycle (Fig. 2a–d). Conversely, CD317 had only marginal effects (~2-fold) on HIV-1(WT) yield. Viral Gag protein expression in HIV-1(WT)- or HIV-1(delVpu)-infected 293T or HT1080 cells was equal in the presence or absence of CD317 (Fig. 2c, d), indicating that all preceding steps in HIV-1 replication were unaffected and that CD317 specifically inhibited HIV-1 virion release. In addition, transfection of 293T cells with vectors expressing MLV GagPol revealed that stable expression of CD317 markedly reduced the yield of MLV particles, without affecting MLV Gag expression (Fig. 2e). Moreover, inhibition of MLV particle release by CD317 was almost completely abolished when Vpu was co-expressed with MLV GagPol. (Fig. 2e).

In single-cycle HIV-1 replication assays, conducted in HeLa cells that constitutively express CD317, HIV-1(delVpu) virions were released approximately 20-fold less efficiently than HIV-1(WT) counterparts (Fig. 2f, g). To determine whether CD317 was required for this phenotype, the same assay was done using HeLa cells transfected with a *CD317*-targeted short interfering RNA (siRNA) pool that effectively knocked down expression of the corresponding haemagglutinin (HA)-tagged protein (Supplementary Fig. 1), or a control siRNA pool. *CD317*-targeted siRNAs, but not control siRNAs, enhanced the yield of HIV-1(delVpu) from HeLa cells by ~13-fold, but had negligible effects on HIV-1(WT) yield (Fig. 2g). Notably,

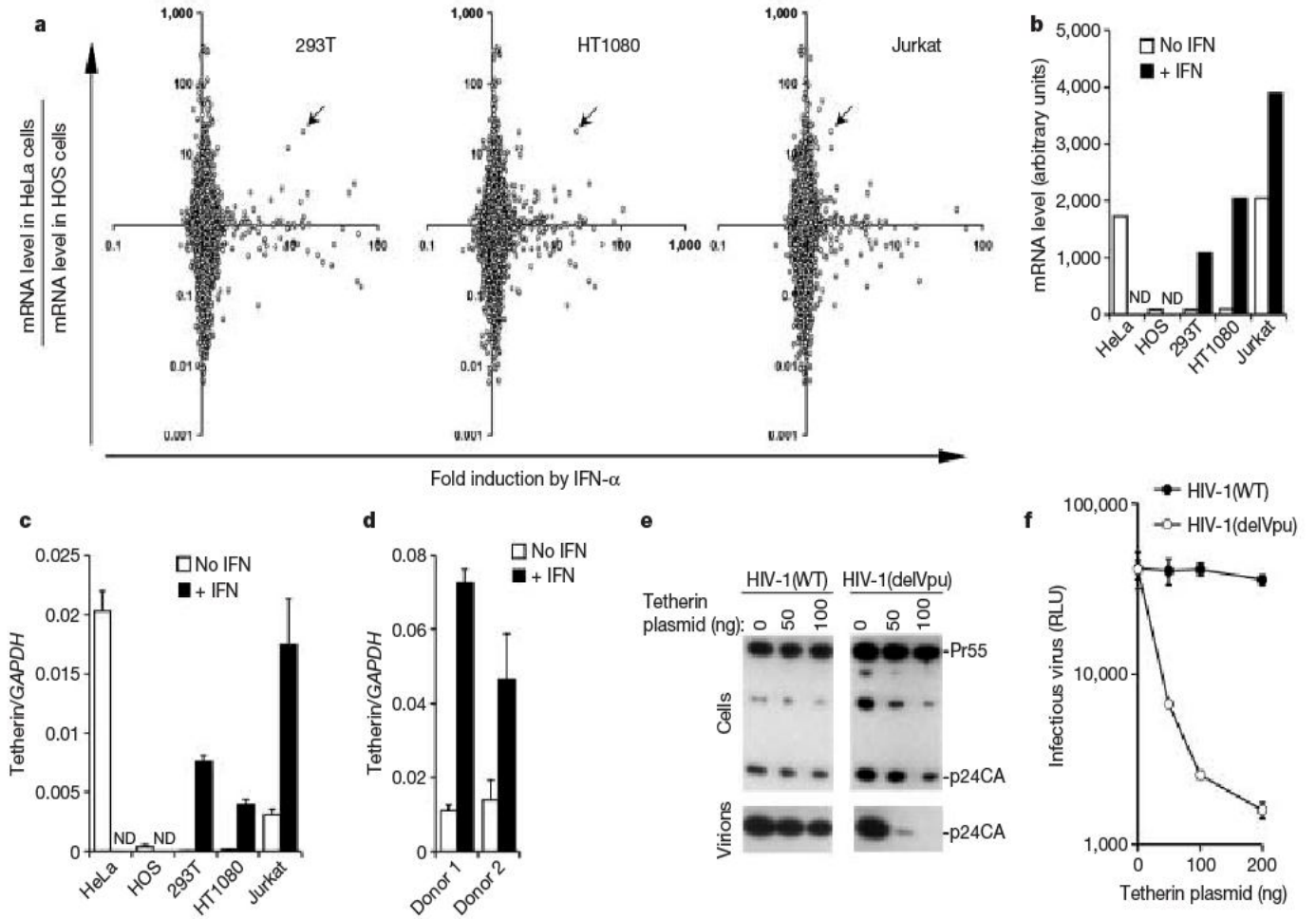


Figure 1 | Identification of CD317 as a candidate tetherin. **a**, Gene expression analysis of cells in which Vpu is, or is not, required for HIV-1 virion release. The ratio of mRNA levels in HeLa cells divided by those in HOS cells (*y* axis) is plotted against levels in IFN-α-treated 293T, HT1080 or Jurkat cells divided by those in corresponding untreated cells (*x* axis). Candidate tetherins appear in the upper right quadrant; arrows indicate *CD317*. **b**, levels of *CD317* mRNA (arbitrary units) as measured in the array analysis. ND, not done. **c**, **d**, Levels of the candidate tetherin mRNA in cell lines (**c**) or $CD4^+$ T cells (**d**), presented as mRNA copies per copy of *GAPDH* (±s.d., $n = 3$), as measured by qRT–PCR. **e**, Western blot analysis (anti-p24 capsid) of 293T cell lysates and virions after co-transfection with HIV-1(WT) and HIV-1(delVpu) proviral plasmids and varying amounts of CD317 (untagged) expression plasmid. **f**, Same as **e**, except that infectious virus yield was measured using TZMbl indicator cells and expressed as relative light units (RLU) (±s.d., $n = 3$).

CD317 knockdown had no effect on Gag expression and processing (Fig. 2f); rather it relieved a requirement for Vpu during particle release.

CD317 (referred to hereafter as tetherin) has an unusual topology, in that it harbours an amino-terminal cytoplasmic tail, a single-membrane-spanning sequence, a predicted extracellular coiled-coil domain and a putative carboxy-terminal glycosyl phosphatidylinositol (GPI) membrane anchor[15,16] (Fig. 3a). All of the aforementioned assays were done using an authentic, untagged version of tetherin.

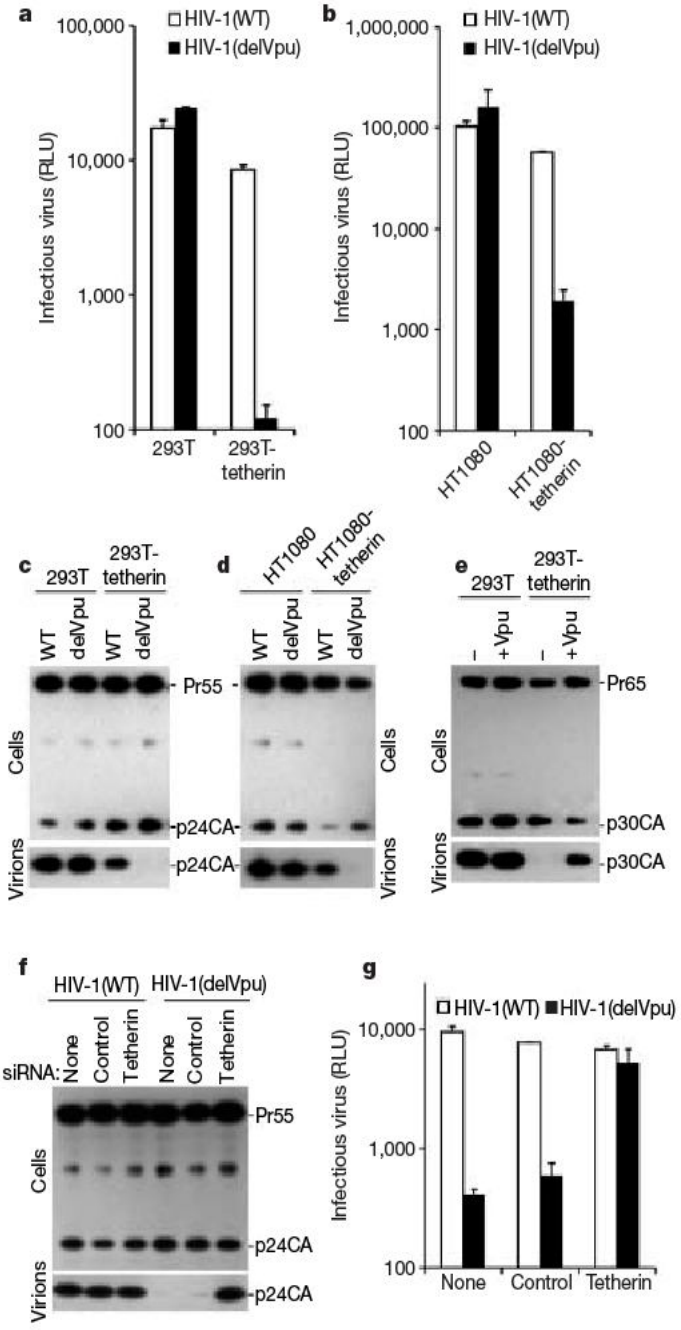


Figure 2 | Tetherin blocks HIV-1 virion release and is necessary to impose a requirement for Vpu. **a**, **b**, Infectious virion yield (±s.d., $n = 3$), measured as in Fig. 1, from 293T cells (**a**) or HT1080 cells (**b**) that were unmodified or stably expressed untagged tetherin, after a single cycle of HIV-1(WT) or HIV-1(delVpu) replication. **c**, **d**, Same as for **a** and **b**, except that western blot analysis of cell lysates along with progeny virions (anti-p24 capsid) was done. **e**, Western blot analysis of cell lysates along with progeny virions (anti-p30 capsid) after expression of MLV GagPol, in the absence or presence of Vpu, in 293T cells that were unmodified or stably expressed untagged tetherin. **f**, Western blot analysis (anti-p24 capsid) of HeLa cells and progeny virions during a single cycle of HIV-1 replication after transfection with no siRNAs (none), control or tetherin-specific siRNAs. **g**, Same as **f**, except that infectious virion yield (±s.d., $n = 3$) was measured, as in Fig. 1.

However, to facilitate more detailed analyses, an N-terminally HA-epitope-tagged tetherin derivative was generated, and this protein accurately recapitulated the antiviral properties of the untagged molecule (Fig. 3b, c). HA–tetherin exhibited a complex pattern of distribution in cells, suggesting that it localized to multiple membranous compartments, including the plasma membrane (Supplementary Fig. 2). Moreover, tetherin migrated as several species in SDS–PAGE analyses, presumably as a result of heterogeneous glycosylation[11] (Fig. 3b). A tetherin variant with a truncated N-terminal cytoplasmic tail (delCT) migrated as a single species in SDS–PAGE analyses, and its subcellular distribution was aberrant, suggesting that it remained non-glycosylated or uniformly glycosylated, and failed to complete proper transport through the secretory pathway (Fig. 3b and Supplementary Fig. 2). Notably, tetherin(delCT) did not affect HIV-1(delVpu) particle release (Fig. 3b, c). Removal of the putative GPI modification signal in tetherin, (delGPI), increased tetherin protein levels but did not overtly alter glycosylation or subcellular distribution (Fig. 3b and Supplementary Fig. 2). However, removal of the putative GPI anchor completely abolished tetherin's ability to inhibit HIV-1(delVpu) release (Fig. 3b, c).

Effects of tetherin on nascent HIV-1 particles

In circumstances where HIV-1 release requires Vpu expression, Vpu-defective HIV-1 virions assemble and mature, but remain tethered to the surface of cells[3,4,6]. Thereafter, a proportion of virions are internalized and accumulate in endosomes[6,9,10]. To determine whether tetherin induced this phenotype, 293T cells were transiently co-transfected with plasmids expressing HIV-1 Gag–GFP, tetherin and/or Vpu. Tetherin markedly increased the proportion of cells that contained prominent intracellular accumulations of Gag–GFP puncta, which were often associated with $CD63^+$ endosomes (Fig. 4a, b and Supplementary Fig. 3a). Conversely, a mutant tetherin (delGPI) that did not inhibit HIV-1(delVpu) release did not affect the distribution of Gag–GFP (Supplementary Fig. 3a). Importantly, tetherin did not induce intracellular accumulation of Gag–GFP when Vpu was co-expressed (Fig. 4a and Supplementary Fig. 3a). Moreover, accumulation of Gag–GFP puncta in the endosomes of 293T cells stably expressing tetherin was blocked by a dominant-negative

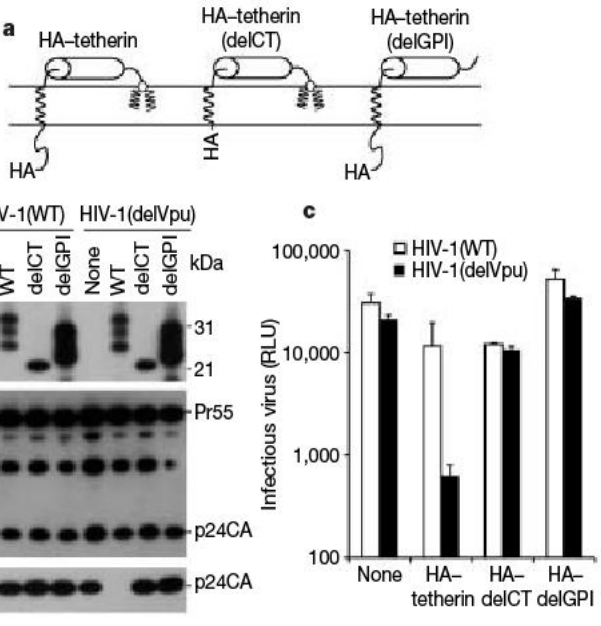
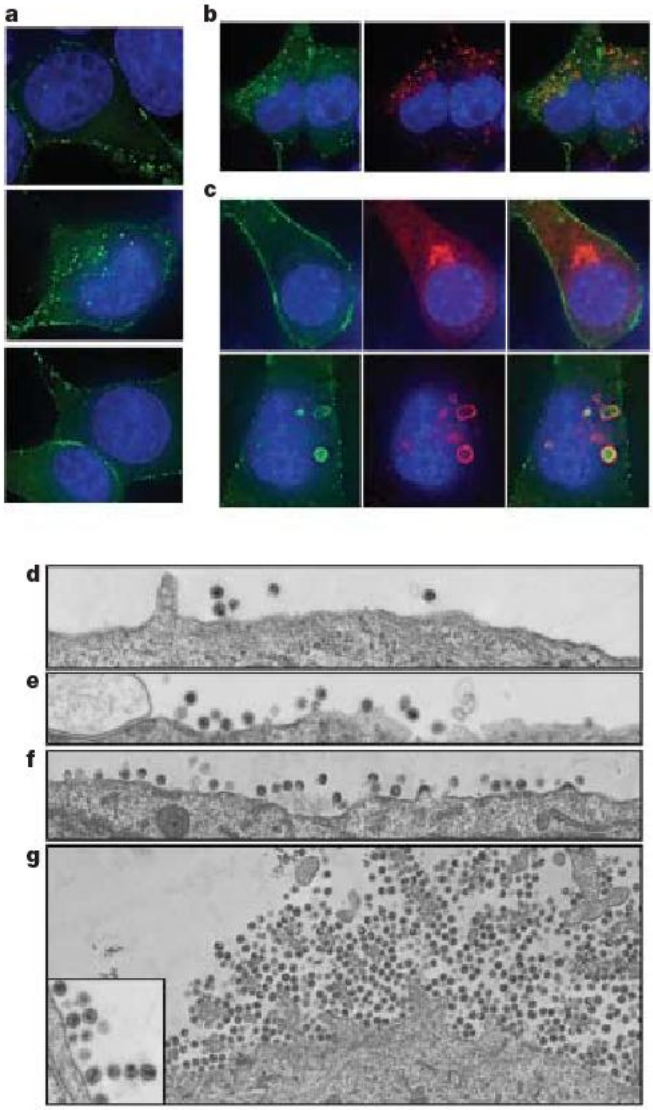


Figure 3 | Effects of HA-tagged tetherin and mutants on HIV-1 release. **a**, Schematic representation of the HA-tagged tetherin and mutants. **b**, Western blot analysis (anti-HA, upper panel; p24 capsid, centre and lower panels) of cells and virions after transfection of 293T cells with proviral plasmids and plasmids expressing HA-tagged tetherin molecules. **c**, Virion yield from the same cells as in **b**, (±s.d., $n = 3$) measured using infectivity assays.

Rab5a mutant (S34N) as well as by Vpu (Fig. 4c and Supplementary Fig. 3b, c), suggesting that tetherin-induced association of Gag–GFP with $CD63^+$ compartments occurred via endocytic uptake of nascent particles from the plasma membrane. Consistent with this notion, when swollen endosomes were induced by expression of a dominant-active Rab5a mutant (Q79L), tetherin induced the appearance of Gag–GFP puncta within the swollen endosomal lumen (Fig. 4c). Similar experiments, done in HT1080 cells stably expressing tetherin and infected with an HIV-1 strain carrying YFP inserted into the stalk region of the Gag matrix domain (HIV/MA–YFP)[17,18], showed that YFP-positive puncta accumulated in intracellular compartments of tetherin-expressing cells, specifically when a Vpu-deleted HIV/MA–YFP virus was used (Supplementary Fig. 3d).

Electron microscopic analysis of unmodified HT1080 cells, infected with HIV-1(WT) or HIV-1(delVpu), revealed occasional budding structures and mature virions associated with the plasma membrane (Fig. 4d, e). In HT1080 cells stably expressing tetherin and infected with HIV-1(WT), virions were found associated with the plasma membrane at marginally greater frequency (Fig. 4f). In stark contrast, HIV-1(delVpu) infection of tetherin-expressing cells often resulted in massive accumulations of mature virion particles on cell surfaces (Fig. 4g and Supplementary Fig. 4). Mature HIV-1(delVpu) virions were also frequently observed in intracellular compartments in tetherin-expressing HT1080 cells (Supplementary Fig. 4), consistent with the notion that the particles retained by tetherin can be endocytosed (Fig. 4b, c).

To determine whether tetherin caused protein-mediated tethering of HIV-1 to cell surfaces, we used an assay in which tethered virions are recovered from cell surfaces by protease 'stripping'[6,8]. In HIV-1-transfected or -infected 293T cell cultures, the majority of virions that can be recovered are constitutively released into the extracellular medium, irrespective of Vpu expression, and only a minor fraction of the total virion output is recovered by protease stripping of cell surfaces[8]. Similarly, In 293T cells stably expressing tetherin, HIV-1 (WT) virions were also predominantly constitutively released and only a minor fraction were recovered by protease stripping (Fig. 4h). Conversely, recovery of the majority of HIV-1(delVpu) virions required protease stripping from the surface of tetherin-expressing 293T cells. In similar experiments, co-expression of Rab5a(S34N) slightly reduced the overall production of HIV-1 (WT) and HIV-1(delVpu). However, presumably because virion endocytosis was blocked (Fig. 4c), the recovery of HIV-1(delVpu) by protease stripping of the tetherin-expressing cells' surface was enhanced and, in fact, equalled the yield of constitutively released HIV-1(WT) virions (Fig. 4h).



Localization of HIV-1 Gag, Vpu and tetherin

Notably, the intracellular compartments in which Gag–GFP or HIV/MA–YFP puncta accumulated in transfection or infection assays were often HA–tetherin-positive (Fig. 5a and Supplementary Fig. 5). Moreover, YFP puncta that were localized at the surface of HIV/MA–YFP(delVpu)-infected cells were also often positive for HA–tetherin (Supplementary Fig. 5). It is possible, therefore, that tetherin is directly responsible for the retention of nascent virions on cell surfaces and their movement into intracellular compartments. Notably, association of Gag–GFP or HIV/MA–YFP puncta with HA–tetherin was prevented by co-expression of Vpu (Fig. 5a and Supplementary Fig. 5), and Vpu itself co-localized almost perfectly with HA–tetherin (Fig. 5b). Although Vpu did not have overt effects on tetherin levels or subcellular distribution (Fig. 3b, Fig. 5b and data not shown), only a minor fraction of HA–tetherin was associated

Figure 4 | Tetherin expression in the absence of Vpu causes virion retention on cell surfaces and virion endocytosis. a, Examples of Gag–GFP localization in 293T cells when expressed alone (top panel), or co-expressed with HA–tetherin (centre panel) or HA–tetherin and Vpu (bottom panel). **b**, Gag–GFP (green) localization in 293T cells stably expressing untagged tetherin and stained with anti-CD63 (red). **c**, Localization of Gag–GFP (green) and either cherry-FP–Rab5(S43N) (top row, red) or cherry-FP–Rab5(Q79L) (bottom row, red) in 293T cells stably expressing untagged tetherin. In **b** and **c**, the right panel is a merged image. **d–g**, Electron micrographs showing plasma membranes of unmodified HT1080 cells (**d**, **e**) or HT1080 cells expressing untagged tetherin (**f**, **g**) after infection with HIV-1(WT) (**d**, **f**) or HIV-1(delVpu) (**e**, **g**). The inset shows an expanded view of plasma-membrane-associated virions. **h**, Western blot analysis of virions that were constitutively released from 293T cells stably expressing untagged tetherin, or released after buffer wash or subtilisin stripping, after transfection with HIV-1(WT) or HIV-1(delVpu) proviral plasmids, alone or with cherry-FP–Rab5(S43N) co-expression.

with the plasma membrane, thus its potential removal from that site was difficult to evaluate. Notably, an inactive Vpu mutant in which the transmembrane domain was replaced with that of CD8 (Vpu(CD8TM))[6] did not co-localize extensively with HA–tetherin (Fig. 5b). Conversely, a Vpu mutant whose activity was partly impaired by truncation of its cytoplasmic tail at residue 50 (Vpu(1–50), Supplementary Fig. 6) co-localized extensively, albeit incompletely, with HA–tetherin, and caused the distribution of tetherin to be more diffuse and plasma-membrane associated (Fig. 5b). Thus, these results suggest a direct or indirect association between tetherin and Vpu, perhaps involving the Vpu transmembrane domain.

Discussion

Overall, tetherin specifically inhibited retrovirus particle release in the absence of Vpu and its expression precisely recapitulated the characteristic HIV-1 particle release defects that have previously been associated with loss of Vpu function[2–4,6,8,9]. Moreover, tetherin co-localized with Vpu and, when Vpu was absent, virions were associated with tetherin at the cell surface and in intracellular compartments. These findings strongly suggest that a major function of the HIV-1 Vpu protein is to facilitate virus spread by antagonizing tetherin-induced particle retention. Notably, previous work has shown that Vpu can enhance the release of a wide range of retroviruses, as well as Ebola virus, in HeLa cells or other cells treated with IFN-α[4,8], suggesting that tetherin acts in a rather nonspecific way to inhibit enveloped virion release. This prediction is borne out by our findings that tetherin can indeed inhibit the release of MLV as well as HIV-1. At present, it is unclear whether only the human form of tetherin has this antiviral function. Orthologues are present in other mammals, but Vpu is not required for efficient HIV-1 particle release from untreated African green monkey COS cells[5], and fails to stimulate particle release that has been is inhibited by IFN-α treatment[8]. It will be interesting to determine whether these apparently host-species-specific differences in Vpu activity reflect divergence in tetherin sequence and function.

Tetherin is known to be expressed on terminally differentiated B cells[19], bone marrow stromal cells (hence the name BST2)[11] and plasmacytoid dendritic cells[20]. Tetherin has also previously been shown to be broadly expressed on IFN-α induction[19,20]. Because tetherin is GPI-modified, it may partition into cholesterol-rich domains on the plasma membrane[15]. Potentially, this property could drive or facilitate encounters with assembling HIV-1 (or other enveloped virus) particles, which have a similar lipid composition[21]. Although we have not yet determined whether tetherin is actually incorporated into virion particles, the fact that electron microscopic analysis shows that, in the absence of Vpu, HIV-1 particles appeared to be tethered to each other as well as to cells suggests that this may be the case. Moreover, given tetherin's unusual topology, as well as its propensity to dimerize[19], several possible configurations could be imagined in which tetherin might nonspecifically tether virions to cell membranes, and to each other (Supplementary Fig. 7).

Interestingly, tetherin protein levels are decreased by expression of the Kaposi's sarcoma herpesvirus K5 protein[22]. This fact, and the observations that tetherin inhibits divergent retroviruses and is widely expressed in the presence of IFN-α[20], raises the possibility that tetherin might be an important component of the broad innate IFN-α-induced antiviral defence against enveloped viruses. Future work will determine the spectrum of viruses against which tetherin is active, precisely how tetherin induces virion retention, and how its action is counteracted by the HIV-1 Vpu protein.

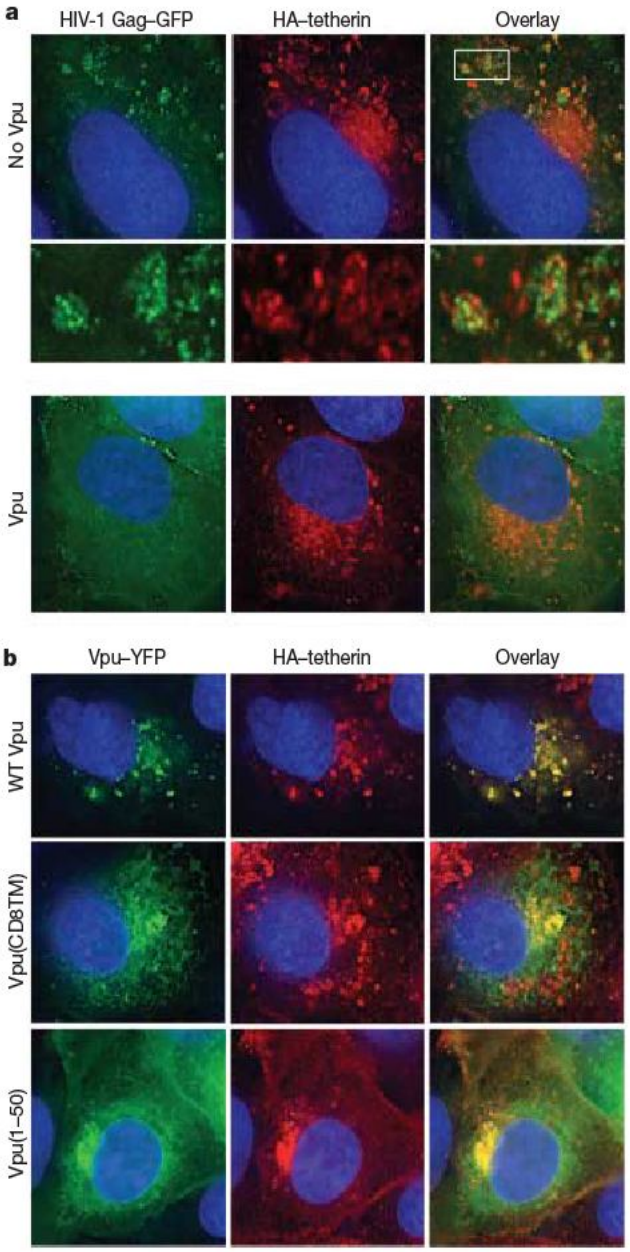


Figure 5 | Tetherin localization in relation to HIV-1 Gag and Vpu. **a**, Localization of transiently expressed Gag–GFP and stably expressed HA–tetherin in HT1080 cells in the absence (top panels) or presence (bottom panels) of co-expressed Vpu. An expanded view of a portion of the top panel (white box) is also shown (centre panels). **b**, Localization of transiently expressed wild-type Vpu–YFP (top row), Vpu(CD8TM) (centre row) or Vpu(1–50) (bottom row) and stably expressed HA–tetherin (red) in HT1080 cells.

METHODS SUMMARY

Microarray analyses. Total RNA from the various cell lines that were either untreated or treated with IFN-α was used to interrogate microarrays (Illumina).

qRT–PCR. Total RNA from untreated or IFN-α-treated cells was used to generate cDNA which was analysed using primer pairs for CD317 and GAPDH (Superarray Bioscience Corporation).

Tetherin expression constructs. Tetherin was transiently expressed using pCR3.1/HA-based plasmids or stably expressed using LHCX-based retroviral vectors. The delCT and delGPI variants of tetherin lacked the N-terminal 20 amino acids and C-terminal 19 amino acids, respectively.

Single-cycle HIV-1 replication and transfection-based virion release assays. 293T cells were transfected with wild-type (HIV-1(WT)) or Vpu-deleted (HIV-1 (delVpu)) proviral plasmids, along with tetherin expression plasmids. Alternatively, HeLa cells (that were either untransfected or transfected with siRNAs), 293T cells, or HT1080 cells were infected with VSV-G-pseudotyped HIV-1(WT) or HIV-1(delVpu). Thereafter, culture supernatants and infected cells were collected. Virus particle pellets collected from culture supernatants and corresponding cell lysates were analysed by western blot assays using anti-p24 monoclonal antibodies. Infectious virus yields were determined using HeLa-TZMbl indicator cells.

Protease stripping assays. 293T cells stably expressing tetherin were transfected with proviral plasmids. Constitutively released particles or particles that were

released after buffer wash or subtilisin stripping, as well as corresponding cell lysates, were analysed by western blotting.

Microscopy. Cells, plated on coverslips, were transfected with combinations of plasmids expressing HIV-1 Gag–GFP, tetherin, Vpu, and/or Rab5a mutants. Alternatively, cells were infected with VSV-G-pseudotyped HIV-1/MA–YFP. Anti-HA or anti-CD63 antibodies were used for staining. Microscopy was done using a Deltavision suite. For electron microscopy, HT1080 cells were infected with VSV-G-pseudotyped HIV-1(WT) or HIV-1(delVpu). Sections were stained with uranyl acetate and lead citrate and observed with a FEI G2 Tecnai TEM.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions S.J.D.N. and P.D.B conceived and designed the experiments. S.J.D.N. performed the experiments with assistance from T.Z. P.D.B. wrote the paper.

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ARTICLES

Host genome surveillance for retrotransposons by transposon-derived proteins

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Transposable elements and their remnants constitute a substantial fraction of eukaryotic genomes. Host genomes have evolved defence mechanisms, including chromatin modifications and RNA interference, to regulate transposable elements. Here we describe a genome surveillance mechanism for retrotransposons by transposase-derived centromeric protein CENP-B homologues of the fission yeast *Schizosaccharomyces pombe*. CENP-B homologues of *S. pombe* localize at and recruit histone deacetylases to silence *Tf2* retrotransposons. CENP-Bs also repress solo long terminal repeats (LTRs) and LTR-associated genes. *Tf2* elements are clustered into 'Tf' bodies, the organization of which depends on CENP-Bs that display discrete nuclear structures. Furthermore, CENP-Bs prevent an 'extinct' *Tf1* retrotransposon from re-entering the host genome by blocking its recombination with extant *Tf2*, and silence and immobilize a *Tf1* integrant that becomes sequestered into Tf bodies. Our results reveal a probable ancient retrotransposon surveillance pathway important for host genome integrity, and highlight potential conflicts between DNA transposons and retrotransposons, major transposable elements believed to have greatly moulded the evolution of genomes.

Transposable elements, prevalent in most eukaryotic genomes, exert diverse effects on their hosts, profoundly influencing the organization, integrity and evolution of the host genome, and the host transcriptome[1,2]. Host cells have devised strategies, such as DNA and histone methylation and RNA interference (RNAi), to control transposable element activity[3–6]. Whereas RNAi has a prominent role in silencing transposable elements in most organisms, in mammals, an adaptive RNAi response, thus far, has been observed only in germ cells[7]. Moreover, RNAi in *S. pombe* is known to target preferentially a specific class of repeat (*dg* and *dh*) elements associated with constitutive heterochromatic regions, but RNAi and heterochromatin machineries have minor roles in silencing *Tf2* retrotransposons and solo LTRs[8] dispersed throughout the genome[9,10]. Therefore, additional RNAi-independent mechanisms probably exist to recognize and silence transposable elements.

The long evolutionary presence of transposable elements in eukaryotic genomes has resulted in many instances of hosts 'domesticating' transposable-element-encoded factors to perform cellular functions[11–13]. Human CENP-B protein, which facilitates centromere formation by binding to short repeats within centromeric alpha satellite DNA[14,15], is derived from transposases of pogo DNA transposons[16,17]. CENP-B, highly conserved in mammals, has homologues in other systems[13,18]. The genome of *S. pombe* encodes three CENP-B homologues: Abp1, Cbh1 and Cbh2 (ref. 19). These have been shown to have redundant roles in centromeric heterochromatin formation and chromosome segregation[19,20]. However, unlike cells lacking heterochromatin proteins such as Swi6/HP1, loss of Abp1 results in a slow-growth phenotype exacerbated by additional deletions of other CENP-B homologues[18,19], suggesting additional functions by CENP-Bs.

Here, we report roles for CENP-B homologues in the surveillance for retrotransposons and in genome organization in *S. pombe*. CENP-Bs bind to *Tf2* retrotransposons and their remnants, and mediate *Tf2* silencing by recruiting the class I histone deacetylase (HDAC) Clr6 (refs 21, 22) and the class II HDAC Clr3 (ref. 23). CENP-Bs also repress several genes through nearby LTRs and facilitate HDAC recruitment to heterochromatic loci. We demonstrate that *Tf2* retrotransposons scattered across the genome are clustered into Tf bodies, the organization of which is mediated by CENP-Bs. This study highlights a potentially hitherto unrecognized host genome surveillance mechanism that exploits the targeting of a specific class of transposable elements by proteins of another class to rein in transposable element activities.

Genome-wide distributions of Abp1 and Cbh1

To gain insights into disparate phenotypes exhibited by mutations in the CENP-B family members of *S. pombe*, we mapped the distributions of these factors across the genome. Chromatin immunoprecipitation coupled with DNA microarray (ChIP–chip) analyses revealed Abp1 and Cbh1 binding at numerous loci on all three chromosomes (Fig. 1a). Consistent with the original finding of Abp1 association with certain ARS sites[24], we found Abp1 enrichment at *ars3002* (Supplementary Fig. 1a). Abp1 was also detected at centromeres (Supplementary Fig. 1b)[19,20]. Close examination revealed preferential Abp1 enrichment at sequences immediately outside of *dh* repeats of centromere I and II, although low levels of binding were also observed at other sites (Supplementary Fig. 1b). Notably, Abp1 and Cbh1 were highly enriched at *Tf2* retrotransposons (Fig. 1a). Abp1 and Cbh1 co-localized throughout single, tandem and partial *Tf2* elements with both proteins displaying distinct binding peaks at the 5′ and 3′ LTRs (Fig. 1b and Supplementary Fig. 1c, d), suggesting a possible mode for recruiting these proteins to *Tf2* via specific Abp1/Cbh1-binding sites within *Tf2* LTRs. Conventional ChIP with primers positioned at unique sequences confirmed binding of Abp1 and Cbh1 at all 13 full-length *Tf2* elements and *Tf2* fragment 1 (Fig. 1b and Supplementary Figs 1c, d and 2). We also found that Abp1 and Cbh1 localized to solo LTRs, *wtf* elements (repeats often associated with *Tf* LTRs) and intergenic regions, many of which associate with the promoters of nearby genes (Fig. 1a, c and Supplementary Figs 3 and 4).

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CENP-Bs silence *Tf2* and LTR-associated genes

The binding of CENP-Bs to distinct genomic sites prompted us to investigate whether loss of these factors influences target loci expression. *Tf2* expression was monitored at two sites: LTR, which shares high sequence similarity with some solo *Tf2* LTRs, and coding region, which is present only in full-length *Tf2* and *Tf2* fragment 1 elements. Assays at both sites showed marked increases in *Tf2* expression in an *abp1Δ* strain (Fig. 2a). However, loss of *cbh1* or *cbh2*, or both, did not lead to detectable increases in *Tf2* expression; yet, single or double deletions of these genes in the *abp1Δ* background contributed to a slight further increase in *Tf2* expression relative to the *abp1Δ* mutant strain alone. We extended expression analyses to *wtf* elements and several genes close to solo LTRs enriched for CENP-Bs, and found that similar to *Tf2*, loss of Abp1 accounted for most of the increased levels of expression observed at *wtf* elements and genes associated with LTRs (Fig. 2b, c and Supplementary Fig. 4). These analyses suggest that among the *S. pombe* CENP-Bs, Abp1 is the member critically required for preventing widespread expression of *Tf2*, solo LTRs and LTR-associated genes, and thus could explain the marked growth defect observed in *abp1Δ* cells[18].

Although both Abp1 and Cbh1 co-localize at *Tf2*, Abp1 alone is sufficient to repress *Tf2* expression, suggesting that Abp1 could be responsible for recruiting Cbh1 to *Tf2*. Indeed, the absence of either Cbh1 or Cbh2, or both, had no discernible effect on Abp1 binding to *Tf2* (Fig. 2d), whereas Cbh1, although unaffected by the absence of Cbh2, was delocalized from *Tf2* in cells lacking Abp1 (Fig. 2e). Immunoprecipitation assays showed Abp1 in complex(es) with Cbh1 and Cbh2 (Fig. 2f), supporting the idea that Abp1 recruits Cbh1, and possibly Cbh2, to *Tf2*.

Abp1 recruits HDACs to *Tf2* and a heterochromatic locus

The HDACs Clr3 (a component of the SHREC (Snf2/Hdac-containing repressor complex) silencing complex[23]) and Clr6 (which exists in multi-subunit complexes[21]) have been implicated in controlling *Tf2* expression in *S. pombe*[10,21,23]. We investigated potential genetic interactions between HDACs and Abp1 in *Tf2* silencing. *Tf2* expression was increased and further elevated in *clr6* and double mutant *clr3 clr6* cells, respectively (Fig. 3a)[10]. However, cumulative increase in *Tf2* expression in double HDAC mutant cells was less compared to that observed in *abp1Δ* cells or in cells deficient for Abp1 and either Clr3 or Clr6 (Fig. 3a). These data indicate that Abp1 might act upstream by recruiting these HDACs to silence *Tf2*. Indeed, Abp1 enrichment at *Tf2* was not affected in single or double HDAC mutants, whereas Clr3 and Clr6 localizations at *Tf2* were compromised in *abp1Δ* cells (Fig. 3b, e). Also, Cbh1 localization at *Tf2* was partially affected in HDAC mutant cells (Fig. 3b), suggesting that HDACs might aid in the recruitment of Cbh1 to LTRs. We found Abp1 interacting with Clr3 and Clr6 *in vivo* (Fig. 3c, d). Together, these data show that Abp1 negatively regulates *Tf2* expression, in part, by directly recruiting HDACs to *Tf2*.

We next determined whether Abp1-mediated silencing of retrotransposons intersects with that of HIRA/Hip1 histone chaperone, implicated in *Tf2* silencing[25]. The levels of *Tf2* expression were higher in *abp1Δ hip1Δ* cells compared with single mutant *abp1Δ* or *hip1Δ* cells (Fig. 3f), indicating that Abp1 and Hip1 might silence *Tf2* through distinct pathways. In this regard, Abp1 and Cbh1 localizations at *Tf2* were not affected in *hip1Δ* cells (Fig. 3g).

Heterochromatic silencing also involves HDAC recruitment to the target loci[26]. Previous analysis revealed three major SHREC peaks at the mating-type (*mat*) region[23], two of which appear to correspond to silencer elements near the silent *mat* cassettes (Fig. 4a). The third peak overlaps with CENP-B binding outside of *cenH* (Fig. 4a), a known RNAi-dependent heterochromatin nucleation centre[26], suggesting that in addition to *Tf2*, CENP-Bs might also recruit SHREC at

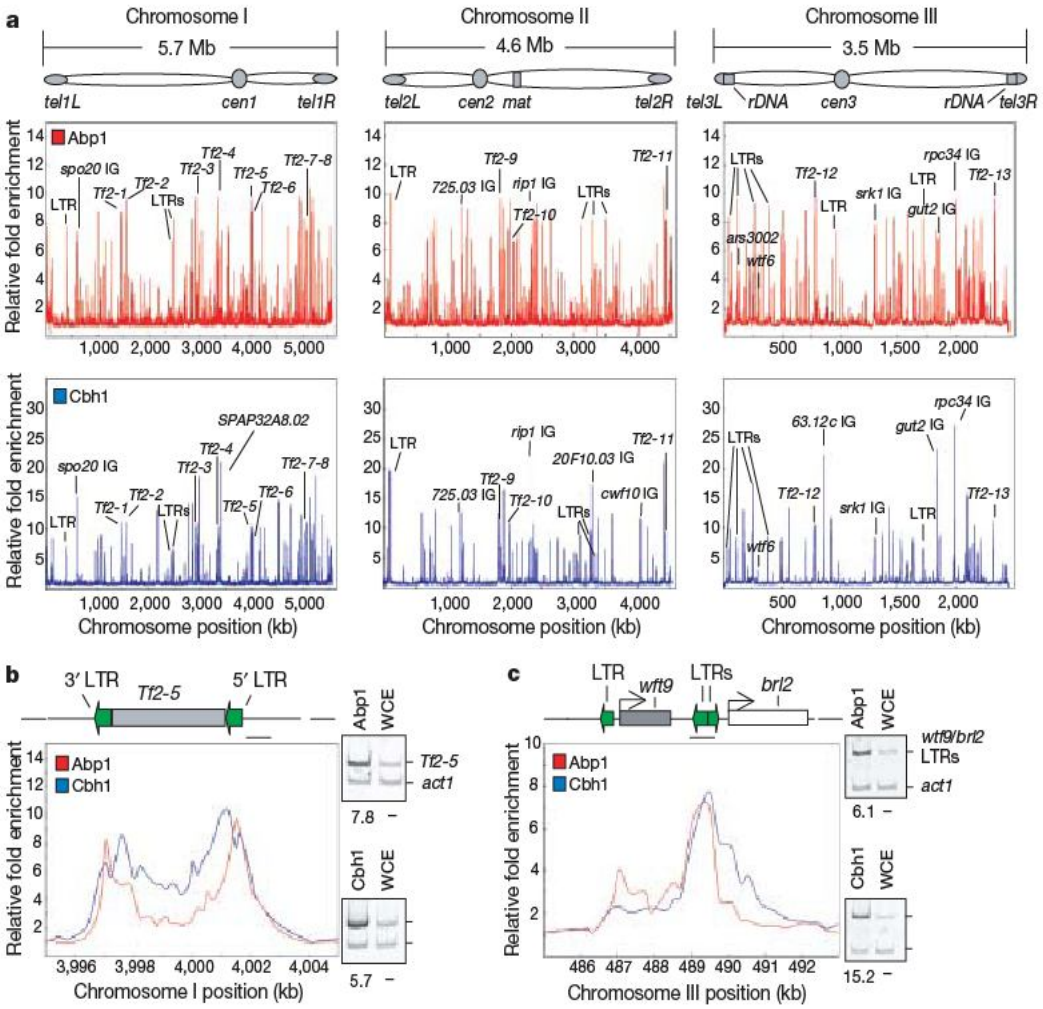

Figure 1 | Genome-wide maps of Abp1 and Cbh1. **a**, Chromosomal distribution profiles of Abp1 and Cbh1. Schematic diagrams of *S. pombe* chromosomes. ChIP assays were performed using strains expressing either Abp1–Flag(3×) or Cbh1–Flag(3×). ChIP–chip relative enrichments of Abp1 and Cbh1 were plotted against respective chromosomal position. Selective binding peaks of Abp1 and Cbh1 are indicated (*IG*, intergenic region; *tel*, telomere; *cen*, centromere; *mat*, mating-type locus). **b**, **c**, Abp1 and Cbh1 co-localize at *Tf* and *wtf* elements. Abp1 and Cbh1 relative ChIP–chip enrichments at *Tf2* and *wtf* elements were confirmed with position-specific primers (black bars) by conventional ChIP PCR (right panels). WCE, whole cell extracts.

mat. Indeed, Clr3 binding was impaired in *abp1Δ* cells specifically at CENP-B binding sites (Fig. 4b). However, considerable levels of Clr3 binding remain across the silent *mat* region in *abp1Δ* cells, probably due to redundant recruitment mechanisms[26].

Dispersed *Tf2* elements cluster into Tf bodies

To gain further insights into the functions of CENP-Bs, we explored their subcellular localization. Immunostaining analyses showed that all three CENP-B proteins display complex nuclear 'network' structures (Fig. 5a and Supplementary Fig. 5a). In addition to localizing to 4,6-diamidino-2-phenylindole (DAPI)-stained nuclear regions, a dense concentration of CENP-Bs could be seen near the nucleolus. The congression of CENP-Bs into nuclear networks prompted us to investigate whether *Tf2* elements are organized into higher-order structures. We tested this by performing fluorescence *in situ* hybridization (FISH) using probes directed against the coding region of *Tf2*. Notably, despite the presence of 13 full-length *Tf2* elements in the genome, only 1–3 *Tf2* foci could be seen per cell nucleus in wild-type cells (Fig. 5b), suggesting that CENP-Bs may have a role in clustering *Tf2* into specialized structures that we refer to as Tf bodies. Indeed, a substantial fraction of single and double mutant CENP-B cells contained more than three *Tf2* spots (Fig. 5b, c).

Mammalian CENP-Bs form homodimers through their conserved carboxy-terminal domain[27,28]. We explored whether Abp1 could form dimers that might contribute to 'bundling' *Tf2* elements into Tf bodies. We found that Abp1 interacts with itself (Supplementary Fig. 6a), and this interaction depends on its dimerization domain. Unlike *abp1Δ*, which affects genomic stability that could potentially contribute to *Tf2* de-clustering, truncation of the Abp1 dimerization domain (*abp1-dm*) did not result in gross defects in growth or chromosome segregation (Supplementary Fig. 6b). Also, *abp1-dm* cells showed little or no change in *Tf2* expression (Supplementary Fig. 6c). However, *abp1-dm* cells were slightly defective in *Tf2* clustering, a phenotype that was further exacerbated when *cbh1* and *cbh2* deletions were combined with *abp1-dm* (Fig. 5d).

De-clustering of *Tf2* elements upon oxidative stress

Host cells subject to stress can activate proviruses and silent transposons[29,30]. *Tf2* expression is known to be upregulated when cells are exposed to oxidative stress[31]. We assessed the effect of oxidative stress on the integrity of Tf bodies. Transient exposure of cells to hydrogen peroxide caused de-clustering of *Tf2* elements, despite CENP-Bs still being bound to *Tf2* (Supplementary Fig. 7a, b). It is possible that signalling pathways required for the cellular response to environmental stresses modify Tf bodies to facilitate rapid restructuring of the genome.

CENP-Bs target an extinct retrotransposon

In addition to *Tf2*, the *S. pombe* genome also contains 249 solo LTRs and LTR fragments, of which 11% belong to an extinct retrotransposon, *Tf1* (refs 8, 32). Because we found high levels of Abp1 and Cbh1 at *Tf1* LTRs, we hypothesized that CENP-Bs could also target *Tf1*. We tested this idea by reintroducing a full-length copy of *Tf1* into the genome. Previous work has shown that *Tf1* can insert into the genome either via integrase-mediated transposition or homologous recombination[33]. Expression of *Tf1-neo* from a plasmid showed a minor increase in *Tf1* transposition in CENP-B mutant cells compared to wild-type cells (Supplementary Fig. 8a). However, we observed a marked increase in frequencies of genomic insertion by *Tf1* in cells lacking CENP-Bs, in particular *abp1Δ* or *cbh1Δ* mutants, when an integrase-defective *Tf1* was used (Supplementary Fig. 8a). This result indicated that Abp1 and Cbh1 could block the homologous recombination of *Tf1*. Sequencing of several *Tf1* integrants from *abp1Δ* and *cbh1Δ* cells showed *Tf1* recombination predominantly with existing *Tf2* elements or their remnants (Supplementary Fig. 8b). Therefore, in addition to silencing, CENP-Bs suppress recombination at *Tf* elements, which has important implications for maintenance of genomic integrity.

We next investigated whether the CENP-B-based surveillance mechanism can recognize a *Tf1* element integrated into the genome. Insertion of *Tf1* at *SPAC7D4.08*, a euchromatic gene, resulted in the targeting of CENP-Bs to this locus (Fig. 6a). CENP-Bs suppress

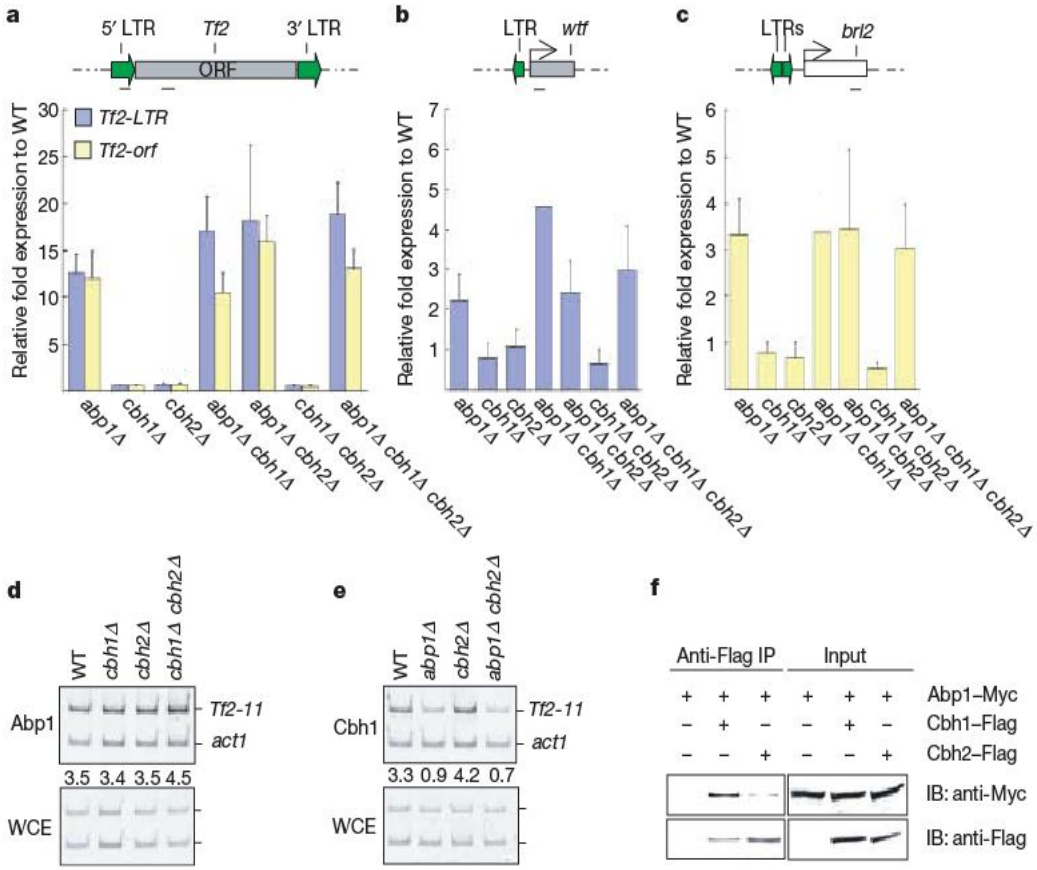

Figure 2 | CENP-Bs silence *Tf2*, *wtf* and several LTR-associated genes. **a**, Increased *Tf2* expression in *abp1Δ* cells. Primers corresponding to LTRs flanking *Tf2* elements (*Tf2-LTR*) or *Tf2* coding region (*Tf2-orf*) were used for qPCR analysis (error bars indicate s.d.; $n = 4$; qPCR primers, black bar). **b**, **c**, Elevated expression of *wtf* (**b**) and *brl2* (**c**) in *abp1Δ* cells (error bars indicate s.d.; $n = 6$). **d**, **e**, Cbh1 localization at *Tf2* is dependent on Abp1 (**e**) but not vice versa (**d**). ChIP analyses of Abp1 and Cbh1 at a *Tf2* element in indicated mutant strains. ChIP fold enrichments relative to *act1* are shown below each lane. **f**, Abp1 interacts with Cbh1 and Cbh2. The input lanes are equivalent to 0.4% of the immunoprecipitation (IP) fraction. The anti-Flag input panel reflects longer film exposure than that of the immunoprecipitation.

transcription and transposition of the *Tf1* integrant, as indicated by increases in expression and transposition frequency of *Tf1* in the *abp1Δ* background (Fig. 6b, c). We also found that insertion of *Tf1* alters the localization of the *SPAC7D4.08* locus in the nucleus. Whereas *SPAC7D4.08* does not usually associate with Tf bodies, *Tf1* insertion at *SPAC7D4.08* resulted in a high incidence of *SPAC7D4.08* co-localizing with Tf bodies (Fig. 6d and Supplementary Fig. 5b), probably as a result of *Tf1* being 'bundled' into Tf bodies.

Discussion

Our study uncovers unexpected targeting of *S. pombe* CENP-B homologues to *Tf* retrotransposons and their remnants to mediate both transcriptional and recombinational repression. CENP-Bs thought to have originated from transposases encoded by an ancient pogo-like DNA transposon[16,17] could hamper the mobility of LTR retrotransposons (this study). Given that both DNA transposons and LTR retrotransposons are flanked by repetitive DNA structures, and transposases bind to terminal inverted repeats of DNA transposons[2], it is possible that during evolution a CENP-B precursor acquired the ability to target retrotransposon LTRs, and subsequently was co-opted by the host into its gene repertoire for controlling transposable elements.

CENP-B-mediated *Tf* silencing is, in part, dependent on CENP-Bs recruiting Clr3-containing SHREC and Clr6 HDAC complexes, which are also required for heterochromatic silencing of centromeric repeats[10,21,23]. Thus, CENP-B localization at heterochromatic regions could aid in silencing by means of HDAC recruitment. Indeed, Abp1 mediates SHREC recruitment to the silent *mat* region. The targeting of HDACs via CENP-B might facilitate assembly of 'closed' chromatin that not only represses transcription but also protects genomic integrity by rendering repetitive sequences recombinationally inert[23,26,34].

Considering that heterochromatin and CENP-Bs recruit a common set of silencing factors, CENP-B regulation of *Tf* elements might represent a 'simple' form of local heterochromatic silencing. In this respect, it would not be surprising that *Tf2* silencing might use other heterochromatin and RNAi components whose normally minor or redundant roles manifest under specific conditions. *S. pombe* CENP-Bs may recruit distinct effectors. Apart from the recruitment of HDACs to *Tf* elements, CENP-Bs might target additional factors critical for transposable element surveillance. As Abp1 and Cbh1 can bind to distinct loci, and that these factors do not always co-localize with SHREC[23], CENP-B binding in contexts other than heterochromatin and transposable elements might recruit activities important for other chromatin transactions, such as transcription and DNA replication.

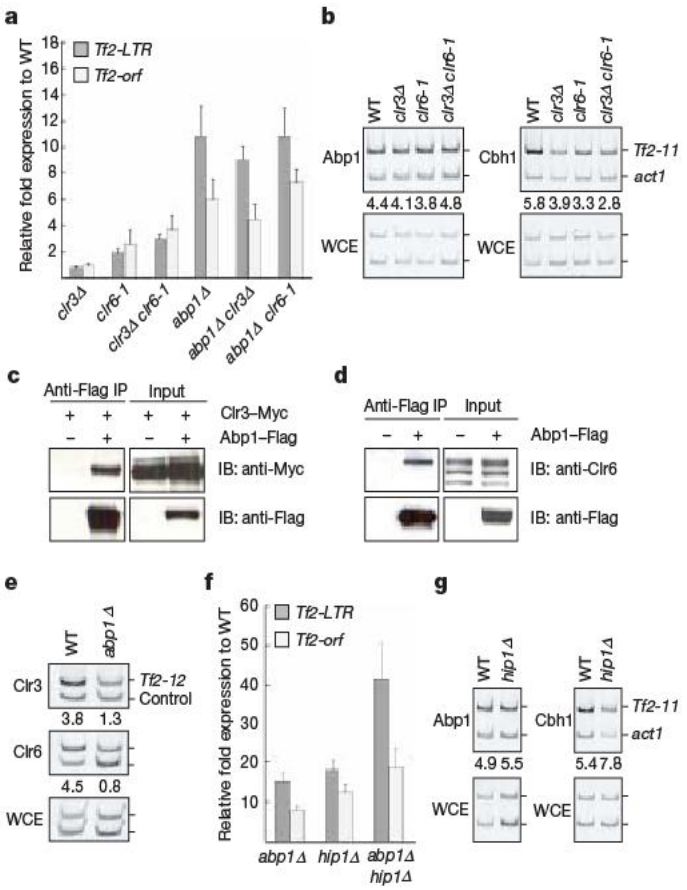

Figure 3 | Abp1 directly recruits HDACs Clr3 and Clr6 and coordinates with Hip1 to silence *Tf2*. **a**, **f**, qPCR analyses of *Tf2* expression in cells carrying single or combinational mutations of *abp1* with HDACs (**a**), or *hip1* (**f**) (error bars indicate s.d.; $n = 6$). **b**, **g**, Effects of mutations of HDACs (**b**) and *hip1* (**g**) on Abp1 and Cbh1 localizations at *Tf2-11* were analysed by ChIP assays. **c**, **d**, Clr3 and Clr6 interact with Abp1. The input lanes are equivalent to 0.1% (**c**) and 0.2% (**d**) of immunoprecipitation fractions. Anti-Flag input panels reflect longer film exposure than that of the immunoprecipitation. **e**, Clr3 and Clr6 localizations at *Tf2-12* in wild-type and *abp1Δ* cells were analysed by ChIP assays. Control corresponds to a gene (*SPBC1348.13*) containing little enrichment for HDACs and CENP-B proteins.

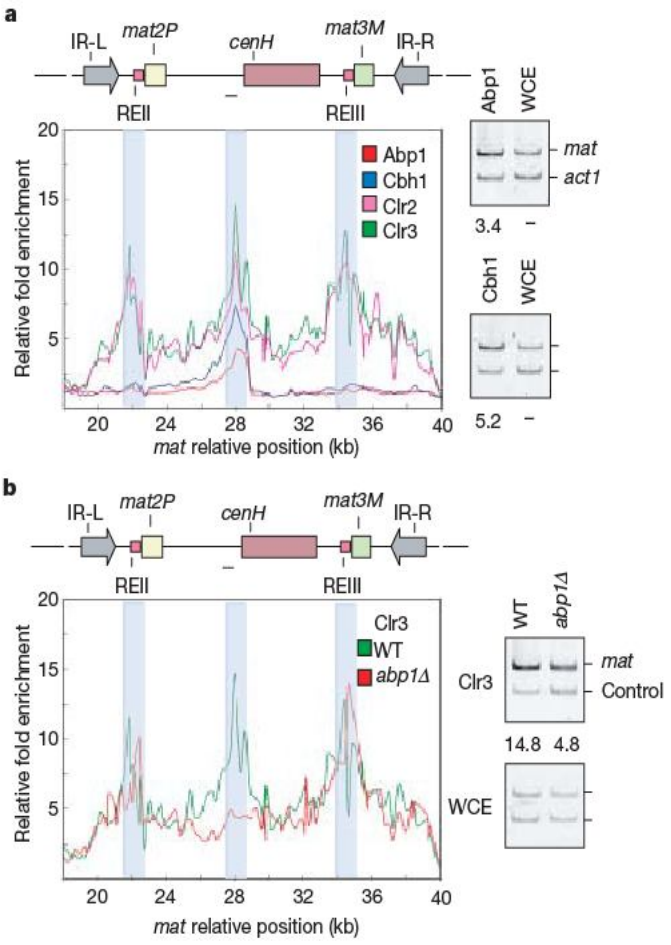

Figure 4 | CENP-Bs recruit the SHREC subunit Clr3 to the silent mating-type locus. **a**, ChIP–chip results of CENP-Bs at *mat* were overlaid with those of SHREC subunits Clr2 and Clr3 (ref. 23), showing their co-localization just outside of *cenH*, an RNAi-mediated heterochromatin nucleation site[26]. Two other SHREC-binding peaks overlap with *mat* silencer elements REII and REIII; the latter contains a binding site for Atf1/Pcr1, involved in Clr3 localization. IR-L and IR-R denote left and right inverted repeat boundary elements flanking the silent *mat* interval, respectively[26]. **b**, Clr3 recruitment at *mat* was impaired in *abp1Δ* cells. Clr3 *mat* distribution in *abp1Δ* cells determined by ChIP–chip was overlaid with that in wild-type cells. Reduced Clr3 binding at *mat* in *abp1Δ* cells and localization of CENP-Bs at *mat* were confirmed by conventional ChIP assays (right panels) with position-specific primers (black bars). Control corresponds to a gene (*SPBC1348.13*) containing little enrichment for HDACs and CENP-B proteins.

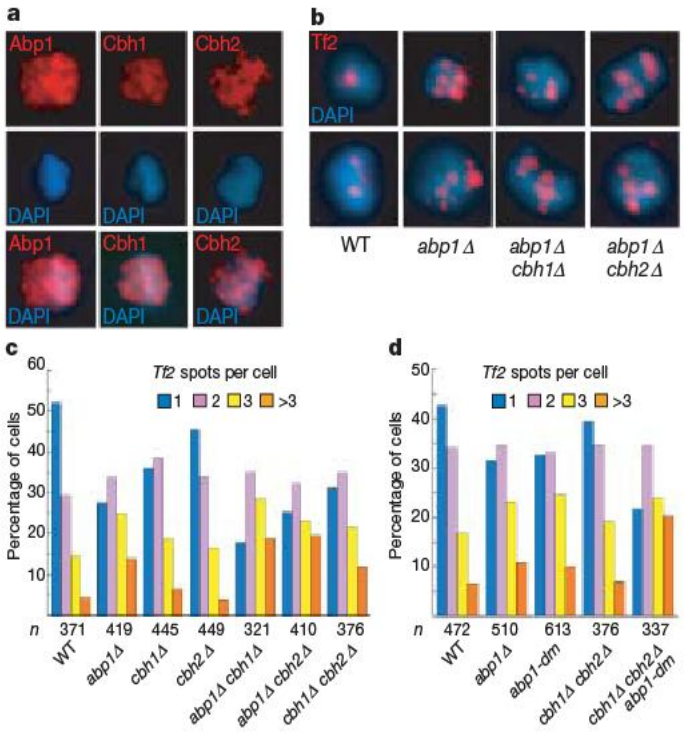

Figure 5 | Discrete CENP-B nuclear distributions and formation of Tf bodies. **a**, Immunofluorescent analyses of Myc-tagged CENP-Bs. **b**, **c**, *Tf2* elements cluster into Tf bodies, and *Tf2* de-clustering is evident in CENP-B mutants. Shown are merged FISH images of *Tf2* and DAPI of two representative cells from each strain. Quantitative FISH analyses (**c**) were performed on the indicated number of cells (*n*) for respective strains. **d**, The Abp1 dimerization domain facilitates *Tf2* clustering. Quantitative FISH analyses of *Tf2* in indicated strains.

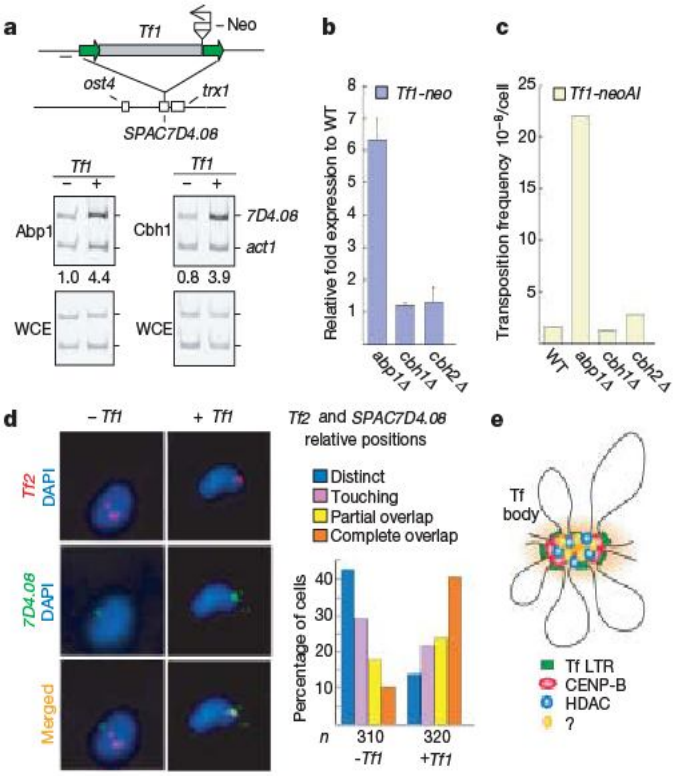

Figure 6 | Genome surveillance controls by CENP-Bs against an introduced extinct *Tf1* element. **a**, Targeting of CENP-Bs to a genomic site containing a *Tf1* integrant. ChIP assays of Abp1 and Cbh1 in strains with or without a *Tf1* integrant at *SPAC7D4.08* is shown. **b**, **c**, Increases in expression (error bars indicate s.d.; $n = 3$) (**b**) and transposition frequency (**c**) of *Tf1* integrant in *abp1*Δ cells. qPCR (**b**) and transposition (**c**) assays (see Methods) of *Tf1* in the indicated mutant strains is shown. **d**, Association of *Tf1* with Tf bodies. Co-FISH analyses with *Tf2*-cy3 (red) and *SPAC7D4.08*-Alexa-488 (green) probes in strains with and without the *Tf1* integrant is shown. **e**, Model of *S. pombe* CENP-Bs in surveillance controls of LTR retrotransposons. CENP-B binding to *Tf* LTRs leads to recruitment of HDACs and presumably other silencing activities that contribute to *Tf* silencing, whereas CENP-B dimerization directly or indirectly promotes *Tf2* clustering.

Accumulating evidence implicates transposable-element-derived sequences as regulators of gene expression in diverse species[1,35–37]. A substantial fraction of human promoters contains transposable element sequences[38], and instances of their contribution to gene regulation have been documented[1,39]. We provide evidence that CENP-Bs bound to LTRs can regulate expression of nearby genes, presumably owing to their ability to recruit chromatin modifiers. CENP-Bs also occupy several gene promoters, that, at one time, might have contained transposable element sequences, but most of which have decayed beyond recognition. Therefore, in addition to retrotransposon surveillance, CENP-Bs and their targeted transposable element sequences might serve as versatile regulatory modules, enhancing the ability of cells to modulate gene regulatory networks[35].

Our analyses revealed unexpected clustering of *Tf2* elements into Tf bodies. These observations are reminiscent of *Drosophila* gypsy retrotransposons brought together into specialized bodies to facilitate chromatin organization[40]. Moreover, transposon-derived MAR/SAR sequences[38] have been shown to create chromatin loops[41]. These findings indicate a conserved role for transposable elements in genome organization. Dimerization of CENP-B proteins[42] (this study) bound to LTRs may directly or indirectly promote either local or long-range loops between LTRs of individual *Tfs* or different *Tf* elements. As such, enrichment of CENP-B proteins at *Tf2* peaks at flanking LTRs but progressively decreases towards the centre of *Tf2* elements. The clustering of *Tfs* into distinct bodies may facilitate transposable element surveillance or other genome-wide processes including prevention of aberrant recombination, coordinated transcriptional control and genome reorganization in response to environmental stresses (Fig. 4g)[29].

This study may have implications for several phenomena observed in other systems. *Drosophila P* and *1360* elements can trigger heterochromatic silencing that is highly dependent on element copy number[43,44]. This mass action requirement for silencing might reflect a high concentration of specific transposable-element-binding proteins similar to CENP-Bs capable of recruiting chromatin modifiers. The formation of Tf bodies could have parallels in mammalian X-chromosome inactivation, in which transposable elements might facilitate the assembly of a silent nuclear compartment[45–47]. Recent evidence suggests that whereas RNAi has a prominent role in silencing transposable elements in germ cells in higher eukaryotes[7], there are probably alternative mechanisms, possibly similar to that of *S. pombe* CENP-B-based surveillance, for regulating transposable elements in somatic cells.

METHODS SUMMARY

Strains were generated by standard yeast methods. ChIP and ChIP–chip were performed as previously described[9]. Quantitative PCR (qPCR) expression analysis was done using a two-step real-time RT–PCR. Mobility of a *Tf1* integrant was assessed using *Tf1-neo* strains carrying an artificial intron (*AI*)[48,49] inserted in the opposite orientation of *neo*, thereby inactivating *neo*. Retrotransposition rate was determined by fluctuation analysis[50] of cells regaining *neo* owing to *AI* loss during *Tf1* retrotransposition. Sequences of primers used are available in Supplementary Table 1.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Microarray data are available at NCBI GEO repository under the accession number GSE9056 and at NCI (http://pombe.nci.nih.gov/genome/). Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.I.S.G. (grewals@mail.nih.gov)

LETTERS

Depth of a strong jovian jet from a planetary-scale disturbance driven by storms

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The atmospheres of the gas giant planets (Jupiter and Saturn) contain jets that dominate the circulation at visible levels[1,2]. The power source for these jets (solar radiation, internal heat, or both) and their vertical structure below the upper cloud are major open questions in the atmospheric circulation and meteorology of giant planets[1–3]. Several observations[1] and *in situ* measurements[4] found intense winds at a depth of 24 bar, and have been interpreted as supporting an internal heat source. This issue remains controversial[5], in part because of effects from the local meteorology[6]. Here we report observations and modelling of two plumes in Jupiter's atmosphere that erupted at the same latitude as the strongest jet (23° N). The plumes reached a height of 30 km above the surrounding clouds, moved faster than any other feature (169 m s^{-1}), and left in their wake a turbulent planetary-scale disturbance containing red aerosols. On the basis of dynamical modelling, we conclude that the data are consistent only with a wind that extends well below the level where solar radiation is deposited.

Jupiter has been intensively surveyed since February 2007 using a battery of ground-based telescopes and the Hubble Space Telescope (HST) during the New Horizons spacecraft passage. Fortuitously, HST images on 25 March 2007 captured the onset of an uncommon planetary-scale disturbance in the peak of the highest speed jovian jet at 23.5° N planetographic latitude, whose eastward velocity is typically between 140 and 180 m s^{-1} relative to the System III radio rotation period[7–11] (Fig. 1). In the peak of the jet, a small, round bright cloud with a size of 500 km (plume B) grew rapidly, implying from mass flux conservation an average vertical velocity of ~1 m s^{-1} (ref. 12). The second plume, plume A, emerged less than 9.26 hours later at a distance of 63,000 km (55° longitude) to the east of plume B. If plume B had triggered plume A, the required propagation velocity of the triggering mechanism would have to be 1.9 km s^{-1} or about 1.5 times the sound speed at 6 bar. Plumes A and B moved with speeds of 169 and 164 m s^{-1}, respectively. A pattern of slower moving dark patches and bright features with velocities in the range of 100 to 150 m s^{-1} (Fig. 2) was shed sequentially in the wake westward of both plumes, forming the north temperate belt disturbance[13–16]. The subsequent mixing of the clouds and aerosols formed a homogeneous and redder north temperate belt (Fig. 1).

The broad wavelength coverage (from the ultraviolet at 230 nm to the thermal infrared at 20.8 μm) allowed us to retrieve the physical properties of the plumes and surrounding clouds before, during and after the disturbance. Two radiative transfer codes were used to match the plumes' reflectivity and radiance—one for the visible and near-infrared (230 nm–3 μm) to retrieve cloud properties and top altitudes, and the other for the thermal infrared (8.7–20.8 μm) to retrieve temperatures, aerosol opacity and gaseous abundances (Fig. 1e). Particles at the top of the plume heads reached the 60 ± 20 mbar level—that is, about 15 km above the tropopause (which is located between 100–150 mbar) and 30 km above the 200–400 mbar layer (where a tropospheric haze containing the cloud features seen in the visible has its greatest altitude). Above the clouds, a thin stratospheric haze with a base at 40 ± 10 mbar extends upward, but apparently the plumes did not reach this haze. The plumes had high optical thickness (above 50), and best-fitting models give bright particles with a typical radius of 7 ± 1 μm (Supplementary Information). The 200–400 mbar tropospheric haze is formed by smaller particles (about 0.5 μm). During the disturbance, the tropospheric haze increased its optical thickness from 5 ± 1 to 10 ± 2, without changing its altitude significantly, but producing redder aerosols. We therefore conclude that the plumes' tops (Fig. 1d) are formed by fresh icy particles (probably a mix of Jupiter's condensable compounds: water, ammonia and NH_4SH) injected high by the virulent upwelling, and that the disturbance increased the aerosol density and formed a red chromophore—or mixed in a pre-existing chromophore.

The high temporal sampling of our observations allowed us to track (with unprecedented resolution) the changes in the jet velocity profile associated with the disturbance at cloud level: we did this for the day the disturbance erupted (25 March), during its development (March–May) and after its cessation (5 June). (The resolution ranged from 170 to 135 km per pixel on HST frames; Fig. 2.) Disturbance features from 22° to 26° N showed zonal velocities lower than the pre-disturbed jet (Fig. 2a), while the plumes moved faster than the jet peak. We interpret the pattern as the result of instability in the strongly meridionally sheared jet triggered by the rapidly moving bright plumes. The jet became more symmetric after disturbance cessation and the peak velocity decreased by only about 15 m s^{-1}

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(Fig. 2b, c), smaller than the change of ~40 m s^{-1} observed between 1979 and 2000[7–11]. Our conclusion is that the jet remained robust against the turmoil generated by the disturbance evolution, suggesting that it extends deep below the upper clouds where motions are measured.

To test this hypothesis, we ran two different types of dynamical models. The first simulated plume formation using a three-dimensional moist convection code[17,18] (Fig. 3a, b), exploring the atmospheric conditions that can lift the plumes to the high altitudes observed. Previous extensive simulations showed that solar water abundance and high humidities can drive storms to the tropopause but not higher[17], and only if they develop in an environment that is not moist adiabatic[19]. To reach the 60-mbar level, we tested variations in the standard vertical temperature–pressure profile[20,21], and in ammonia and water abundances. A single updraft cell powered by moist water convection (cloud base level at 5–7 bar) requires 2–3 times solar water abundance with nearly 100% relative humidity and with a thermal profile 3–5 K colder than the reference temperature in the upper troposphere (at 200 mbar, Fig. 3b). The plumes might be formed by clusters of these cells[22]. Accordingly, the plumes are deeply rooted and extend vertically more than 120 km (from 60 mbar to 5–7 bar). Their fast zonal motion (169 and 165 m s^{-1}) reflects that of the wind at their base at 5–7 bar. Comparing the velocities at 0.3–0.4 and 5–7 bar indicates that the vertical wind shear below the upper cloud is low (increasing at about 4 m s^{-1} per scale height of 20 km), consistent with the lifetime of the plumes. Under a strongly vertically sheared flow, our model shows that the upwelling is interrupted and the plumes do not develop. The results of the three-dimensional model were injected into a two-dimensional model reproducing the tail observed at the visible cloud level (Fig. 3c inset)[22].

A second test of the vertical wind shear used the EPIC[23] general circulation model to simulate the structure and motions of the disturbance turbulent patterns (Fig. 3c). We used as an input the jet profile that was measured before the disturbance eruption (Fig. 2a). We tested the formation of the turbulent pattern by introducing a point heat source in the jet peak[24] on an atmospheric channel centred at the jet peak with two free parameters: the pressure level of the inflexion point where the vertical wind shear changes, and the value of this vertical wind shear. Above the clouds (from 0.6 to 0.01 bar), we assumed that the jet speed decreases as observed during the Cassini fly-by[21]. The formation of the turbulent pattern is very sensitive to the jet shape at cloud level. We tested this sensitivity by performing the numerical simulations with wind profiles measured at different epochs. Among them, the Cassini wind profile[11] and the 5 June HST profile were stable against the heat source perturbation. On the contrary, the jet profile we measured on the outbreak day (Fig. 2a) becomes rapidly unstable as we introduce the perturbation. Moreover, the disturbance pattern resembles the observed one when the inflexion point is at a pressure of 600 mbar (at the predicted

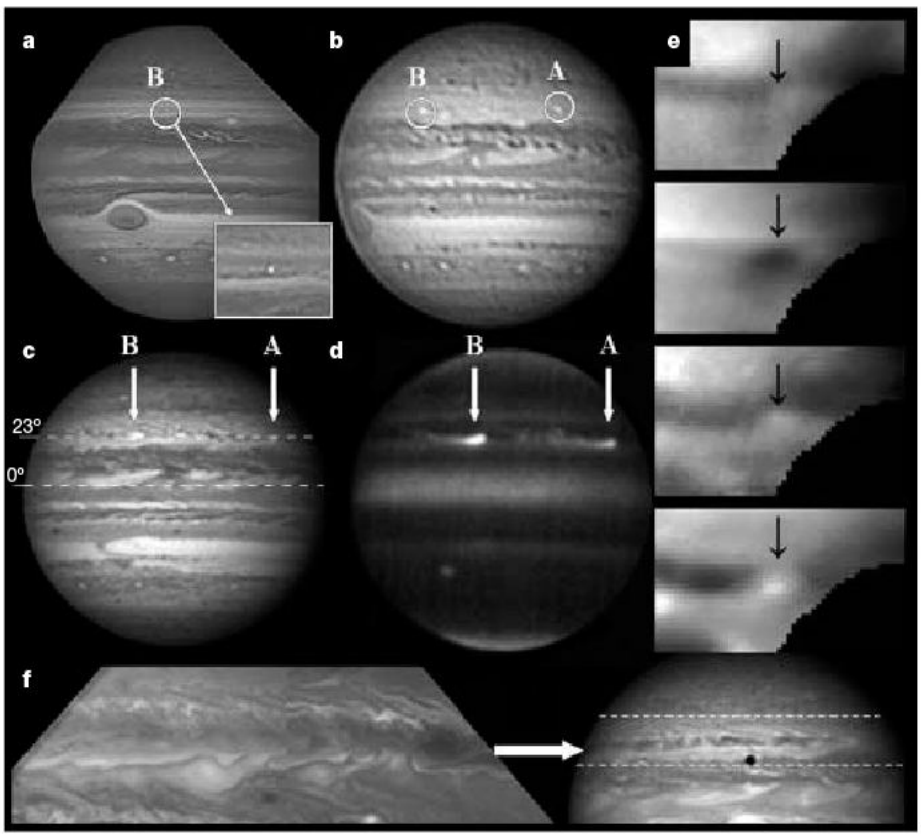

Figure 1 | Plume onset, growth and disturbance development (March–May 2007). **a**, Onset of plume B, 25 March (HST, 410 nm). **b**, Growth of plumes A and B, 27 March (IOPW, visible). Both plumes grew in ~1.3 days to a size of 2,000 km. Plume A was at 23.4° ± 0.5° N and moved with a speed of 169.2 ± 0.5 m s^{-1}, and plume B was at 23.1° ± 0.5° N and moved with a speed of 164.3 ± 1.7 m s^{-1} (see Supplementary Information). **c**, Plumes and disturbances in their wake, 5 April (IOPW, visible). Both plumes were bright features in the visible (meridional extent of 2,500 km, 4,000 km zonal) and particularly prominent at wavelengths sensitive only to high-altitude levels. **d**, Plumes and their tails retrieved at high altitude (NASA-IRTF, 2.3 μm methane filter). **e**, Maps of plume A observed in the thermal infrared on 5 April (IRTF). These excerpts from cylindrical maps show the deviation from the zonal mean of the indicated parameters in the plume head region (top to bottom): temperature at 123.4 mbar, temperature at 393 mbar, aerosol content above 600 mbar and ammonia abundance near 300 mbar. The plume produced lower temperatures near 392.9 mbar, but marginally higher temperatures at 123 mbar, as well as increased ammonia-cloud level aerosols and 300-mbar ammonia gas abundances. The arrows mark the plume head region. **f**, Turbulence and wavy patterns in the wake of plume A followed by the formation of a new north temperate belt as observed on 1 May by the Hubble Space Telescope (left, 410 nm, magnified view) and IOPW observers (right; the horizontal bars limit the area of the north temperate belt). The HST image shows the turbulent pattern formed at latitudes 20–28° N, with a predominant wavelength of 7,000–12,000 km. Plume B disappeared around the time the dark patches shed by plume A reached the location of plume B in about 14 days. The disturbance advanced to the west, encountering plume A on its eastern side on 11 May (its lifetime was 45 days), when it disappeared.

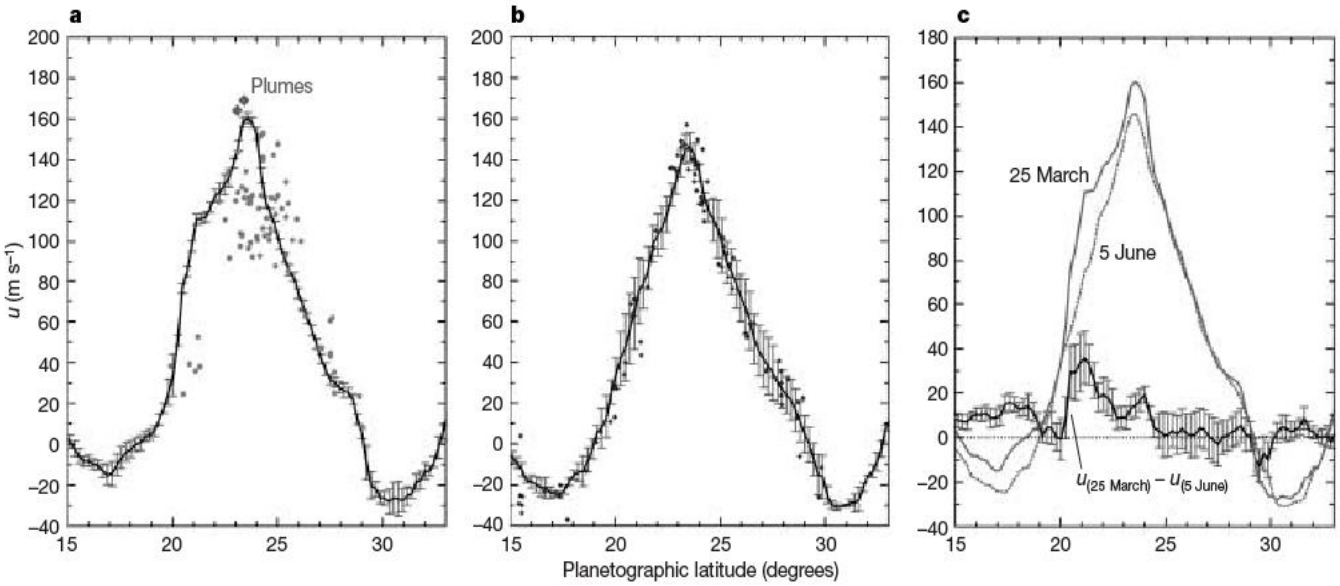

Figure 2 | Time dependence of the profile of the north temperate jet. **a**, Zonal velocity of the disturbance features (filled circles) and plumes A and B (filled diamonds) between 25 March and 31 May 2007 measured by cloud tracking, as compared to the profile the day of the eruption (continuous line, pre-disturbance stage on 25 March 2007) obtained by one-dimensional zonal brightness correlation on HST images (error bars represent the standard deviation of the measurements). **b**, Jet profile after the disturbance ended, 5 June 2007. The continuous line represents the profile obtained by zonal correlation and the dots by cloud tracking, both on HST images (error bars represent the standard deviation of the measurements). **c**, Comparison of the jet profiles before the eruption (25 March 2007) and after the disturbance end (5 June 2007). The difference between both profiles as a function of latitude is shown as a darker line with associated error bars representing the standard deviation of the measurements.

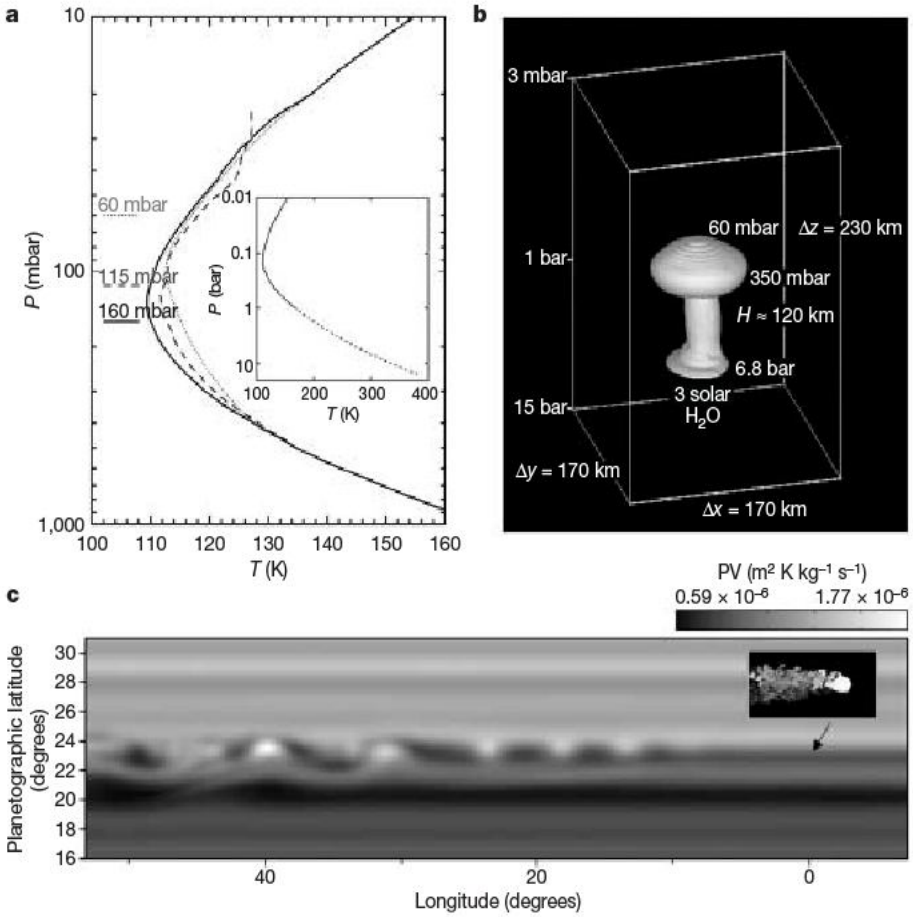

Figure 3 | Models of the plume onset and disturbance development. **a**, **b**, Wet convective three-dimensional model of the plumes. **a**, Thermal profiles used to run the simulations and the cloud top level reached by the convective cell assuming a deep water content of 3 times solar abundance and 95% of relative humidity above the condensation level. *P*, pressure; *T*, temperature. The continuous line corresponds to the Cassini CIRS thermal profile at the NTB location[19], the dashed line to the Voyager IRIS thermal profile[18] and the dotted line to a synthetic profile with less static stability from 500 to 200 mbar required for the storms to reach the 60 mbar level. The inset shows the wet adiabat extension deep in the atmosphere. **b**, Convective cell resulting from the model able to fit the observed cloud tops of the plumes and the domain of simulation. **c**, The plume brightness distribution (inset) results from a two-dimensional model of a round cloud placed in the peak of the jet and evolving as it interacts with the meridional shear of the zonal wind[17] with a spatial resolution of 5 km over a 10,000 × 5,000 km area. The map (main panel) shows the distribution of Ertel's potential vorticity (PV; greyscale) at 650 mbar after 30 days for a simulation where the jet extends vertically downwards with constant value from the upper cloud layer at altitude ~0.6 bar down to at least 5–7 bar (the location of the water clouds and the plume source). Inset and map are at the same scale.

ammonia cloud level) and the vertical wind shear down from this altitude to the water cloud base at 5–7 bar is zero or increases slightly with depth. This result reinforces the conclusion that the jet extends with low vertical wind shear down along the whole 'weather layer' to at least the 5–7 bar pressure level.

Our 5–7 bar zonal wind speed of 169–165 m s^{-1} at 23° N is consistent with the 180 m s^{-1} velocity determined for that depth by the Galileo probe[4] at 7° N, as well as the 240 m s^{-1} wind speed from previous EPIC simulations of the long-term behaviour of the 1990 disturbance[24]. These studies agree that the winds do not decay below the upper ammonia cloud, and extend deeper than the solar radiation penetration level at 1–2 bar (see Supplementary Information), in agreement with previous ideas on the depth and stability of the jovian jet structure[25].

A comparison of this disturbance with two previous events in 1975 and 1990 suggests additional caveats and surprising similarities[13,14]: first, the outburst occurred with an interval of ~15–17 years; second, the plumes always appear in the jet peak within ±0.5°; third, the disturbance erupted with two plumes (not one, three or more); fourth, the eastern plume, located in longitude east of the other (taking as a reference the shortest distance between both) is always the fastest; and last, the plumes moved with a speed in the range ~165–170 m s^{-1} in all three events.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

Optical pumping of a single hole spin in a quantum dot

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The spin of an electron is a natural two-level system for realizing a quantum bit in the solid state[1–16]. For an electron trapped in a semiconductor quantum dot, strong quantum confinement highly suppresses the detrimental effect of phonon-related spin relaxation[1–7]. However, this advantage is offset by the hyperfine interaction between the electron spin and the 10^4 to 10^6 spins of the host nuclei in the quantum dot. Random fluctuations in the nuclear spin ensemble lead to fast spin decoherence in about ten nanoseconds[8–14]. Spin-echo techniques have been used to mitigate the hyperfine interaction[14,15], but completely cancelling the effect is more attractive. In principle, polarizing all the nuclear spins can achieve this[16,17] but is very difficult to realize in practice[12,18,19]. Exploring materials with zero-spin nuclei is another option, and carbon nanotubes[20], graphene quantum dots[21] and silicon have been proposed. An alternative is to use a semiconductor hole. Unlike an electron, a valence hole in a quantum dot has an atomic *p* orbital which conveniently goes to zero at the location of all the nuclei, massively suppressing the interaction with the nuclear spins. Furthermore, in a quantum dot with strong strain and strong quantization, the heavy hole with spin-3/2 behaves as a spin-1/2 system and spin decoherence mechanisms are weak[22,23]. We demonstrate here high fidelity (about 99 per cent) initialization of a single hole spin confined to a self-assembled quantum dot by optical pumping. Our scheme works even at zero magnetic field, demonstrating a negligible hole spin hyperfine interaction. We determine a hole spin relaxation time at low field of about one millisecond. These results suggest a route to the realization of solid-state quantum networks[24] that can intra-convert the spin state with the polarization of a photon.

Our scheme to initialize a single hole spin is presented in Fig. 1. The quantum dot contains a single hole. The strong in-built strain in an InAs quantum dot shifts the valence light hole states with spin $J=3/2$, $J_z=\pm 1/2$ away from the fundamental gap such that the uppermost valence states have heavy hole character with spin $J=3/2$, $J_z=\pm 3/2$. The corresponding hole spin states are represented as $|\Uparrow\rangle$ and $|\Downarrow\rangle$. A σ^+ -polarized laser drives the $|\Downarrow\rangle$ hole to an exciton state with spin $S_z=-1/2$, $|\Uparrow\Downarrow,\downarrow\rangle$, containing a spin-up, spin-down hole pair and a spin-down electron. Unlike the hole spin, the electron spin interacts with the nuclear spins through the contact hyperfine interaction. The electron spin experiences a small magnetic field, ~20 mT (refs 8–12), as a result of the incomplete cancellation of the random nuclear spins in the quantum dot. The component of the magnetic field in the plane, B^{xy}_{nuclei}, causes the electron spin in the excited state to precess with a period of ~1 ns. The coherence of the precession is destroyed by spontaneous emission with a characteristic time of ~1 ns and results in the shelving of hole spin in the $|\Uparrow\rangle$ state, which does not couple to the laser field, according to the spin selection rule. This process initializes the hole spin with high fidelity provided that the hole spin relaxation time is sufficiently large. Rather than a hindrance, the hyperfine interaction between the electron spin and nuclear spins is beneficial in this scheme because it enables fast hole spin initialization. The scheme works even when the spin states are degenerate. This is advantageous because hole spin flip processes involving a single phonon are turned off in the limit of zero magnetic field[22]. The overall scheme depends on stringent requirements: a very clean optical selection rule, slow hole spin relaxation, and negligible interaction between the hole spin and the nuclear spins. We report here successful implementation of this concept with very high fidelity even at zero applied magnetic field where the hole spin is shielded from the laser only by its spin and not by an energy detuning. We confirm our interpretation by applying a magnetic field along the quantization axis z which slows down the precession, gradually turning off the hole spin initialization process.

Our sample, grown by molecular beam epitaxy, consists of InGaAs quantum dots embedded in the intrinsic region of a metal–insulator–semiconductor field electron transistor (Fig. 2a), allowing deterministic charging. The quantum dots are separated by 25 nm from a hole Fermi sea. With an applied bias between a semi-transparent Schottky gate and the Fermi sea, the quantum dot energy can be controlled relative to the Fermi energy thus determining the charge state. Initial characterization is carried out using photoluminescence spectroscopy (Fig. 2b). Each discrete jump in the emission spectrum signals a charging event and we can identify the charge of each state by the characteristic fine structures[25,26]. The overlap in gate voltage for different charge states is a consequence of the relatively large hole tunnelling time, which we estimate to be ~10 ns, compared to the radiative emission lifetime of 0.8 ns. This is crucial: it ensures that the cotunnelling process[6,7,27] of hole spin relaxation through spin-swap with the Fermi sea is sufficiently weak to implement our scheme.

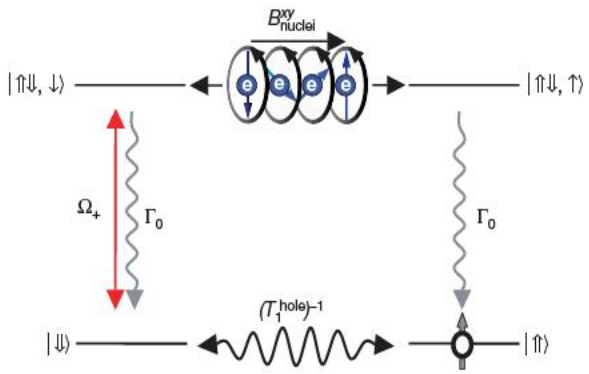

Figure 1 | Scheme to initialize a single hole spin at zero magnetic field. The two hole spin states, $|\Downarrow\rangle$ and $|\Uparrow\rangle$, and the two exciton spin states, $|\Uparrow\Downarrow,\downarrow\rangle$ and $|\Uparrow\Downarrow,\uparrow\rangle$ are depicted with coherent couplings (solid lines) and incoherent couplings (wavy lines). Open (or solid) arrows depict hole (or electron) spins. The dipole transition from $|\Downarrow\rangle$ to $|\Uparrow\Downarrow,\downarrow\rangle$ is driven on-resonance with a σ^+ -polarized laser (Ω_+); electron spin (blue 'e' symbols) precession in the effective magnetic field generated by the nuclear spins coherently couples the $|\Uparrow\Downarrow,\downarrow\rangle$ and $|\Uparrow\Downarrow,\uparrow\rangle$ states. Through spontaneous recombination (Γ_0), the population is shelved in the $|\Uparrow\rangle$ state.

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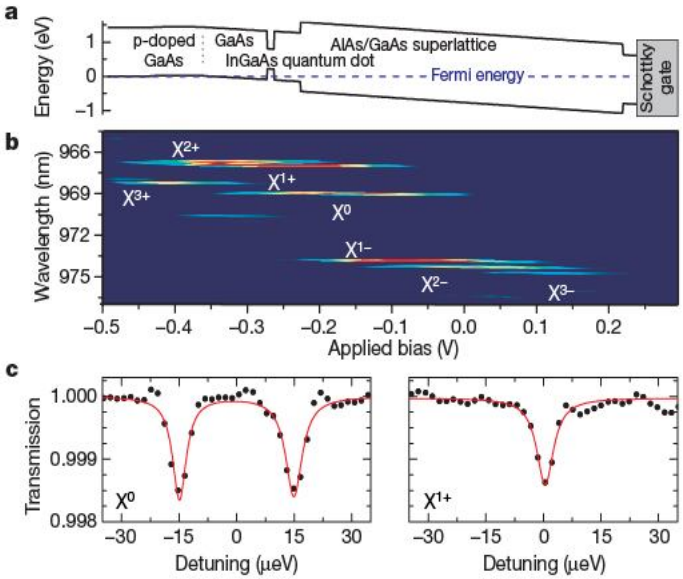

Figure 2 | Experimental methods to probe a single hole spin. **a**, The band diagram for our device. Holes tunnel through a 25 nm barrier into the self-assembled InGaAs quantum dots from the p-doped (carbon doping) back contact. An applied bias between the semi-transparent top gate controls the quantum dot charge state. **b**, Photoluminescence from a single quantum dot shows pronounced Coulomb blockade as the applied voltage is varied. Each spectral jump corresponds to a charging event. **c**, Resonant laser spectroscopy of the empty (X^0) and singly positively charged (X^{1+}) states for the same quantum dot. The detuning is achieved with the applied bias via the Stark effect. The laser has linear polarization oriented at 45° to the crystallographic axis. This polarization equally pumps the two spin transitions for the X^{1+} transition and also the two transitions in the fine structure of the X^0 transition. 1 nW of power is used in each experiment and the sample temperature is 4.2 K. The solid lines are lorentzian fits to the data.

We drive the optical transitions with a highly coherent continuous-wave laser, detecting resonant scattering of the laser light from the quantum dot by measuring the transmission coefficient with very high signal-to-noise ratio[26,28]. Figure 2c displays transmission curves as the quantum dot transition energy is tuned through the laser energy via the Stark effect when the quantum dot is empty (X^0 transition) and charged with a single hole (X^{1+} transition), in both cases using linear polarization. We observe lorentzian lineshapes with linewidths of ~5 μeV. This is larger than both the radiation-broadened linewidth (0.8 μeV) and the linewidth observed in samples with n-doped back contacts (1–2 μeV)[6,7,26,28], but small enough to record high-quality laser spectroscopy data. The increase in linewidth beyond the radiative limit is caused by a spectral fluctuation (see Supplementary Information).

Our main result is revealed in the transmission spectra taken at zero externally applied magnetic field, $B^z_{ext} = 0$, when the two hole spin states are degenerate. We find that the contrast, defined as the size of the transmission dip at zero detuning, is immeasurably small when the exciting laser has circular polarization, either σ^+ (Fig. 3a) or σ^- (Fig. 3b). This suggests that the hole is prohibited by its spin from scattering the laser light, that is, that optical spin pumping is taking place. We prove this by pumping with two lasers with identical wavelength and with the same total power, one with σ^+ and one with σ^- polarization. In this case, a clear transmission dip appears (Fig. 3c). This arises because spin pumping with σ^+ polarization is frustrated by the σ^- excitation, and vice versa, a repumping phenomenon[6].

Hole spin pumping at small magnetic fields is also highly effective. In the Faraday configuration, the degeneracy of the two optical transitions is lifted by the sum of the electron and hole Zeeman energies. When the σ^+ -polarized laser is tuned to the $|\Downarrow\rangle \leftrightarrow |\Uparrow\Downarrow,\downarrow\rangle$ resonance, the contrast is very small, signifying spin pumping into the $|\Uparrow\rangle$ state. Contrast reappears in a two-colour experiment when the σ^- -polarized laser is tuned to the $|\Uparrow\rangle \leftrightarrow |\Uparrow\Downarrow,\uparrow\rangle$ resonance. Repumping is explored more closely in Fig. 3d. Here the maximum contrast is measured as the laser with σ^- polarization is detuned relative to the $|\Uparrow\rangle \leftrightarrow |\Uparrow\Downarrow,\uparrow\rangle$ transition. As expected, maximum contrast is observed when the σ^- laser is on resonance. However, an asymmetry is observed with the repumping signal persisting more at larger than smaller detunings. The spin pumping results demonstrate that the hole spin relaxation time must be much, much larger than the spin

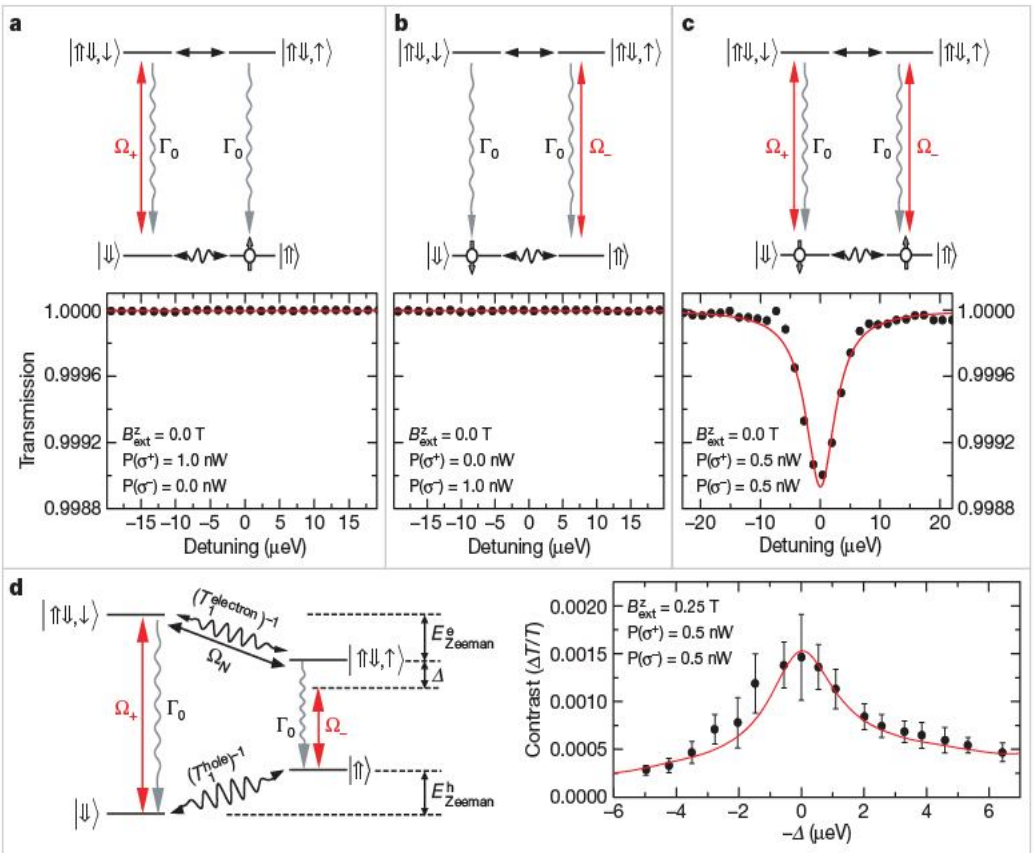

Figure 3 | Experimental demonstration of hole spin pumping. **a**, A laser with σ^+ polarization and power p (σ^+) drives the $|\Downarrow\rangle \leftrightarrow |\Uparrow\Downarrow,\downarrow\rangle$ transition. Any transmission dip lies below the experimental signal-to-noise level. The hole population is shelved in state $|\Uparrow\rangle$. **b**, A laser with σ^- polarization and power p (σ^-) drives the $|\Uparrow\rangle \leftrightarrow |\Uparrow\Downarrow,\uparrow\rangle$ transition. Again, any transmission dip lies below the noise floor. The hole population is shelved in state $|\Downarrow\rangle$. **c**, Simultaneous excitation with both σ^+ and σ^- at the same frequency. A large transmission dip is observed, signifying a frustration of the spin pumping. In **a**–**c**, the solid line is a lorentzian fit to the data. **d**, Two-colour excitation of the Zeeman split transitions ($E^{h,e}_{Zeeman}$, where e or h is for the electron or hole) at $B^z_{ext} = 0.25$ T. The σ^+ laser is on-resonance with the $|\Downarrow\rangle \leftrightarrow |\Uparrow\Downarrow,\downarrow\rangle$ transition while the σ^- laser is swept through the $|\Uparrow\rangle \leftrightarrow |\Uparrow\Downarrow,\uparrow\rangle$ transition. The solid line is the result of the four-level theoretical model using $B^{xy}_{nudei} = 25$ mT and $T_1^{hole} = 250$ μs for this quantum dot. The error bars correspond to s.d. for $n = 10$.

precession time. For the electron spin at fields $B^z_{ext} \leq 0.3$ T, spin pumping is prohibited due to the hyperfine interaction[6,7]. This is not the case for holes: the high fidelity hole spin pumping even at $B^z_{ext} = 0$ demonstrates a negligible hole spin hyperfine interaction.

To investigate the mechanisms involved in the spin pumping process, we measure the contrast as a function of B^z_{ext} for one-colour excitation with both σ^+ and σ^- polarizations (Fig. 4a). The first feature observed is that the contrast increases as B^z_{ext} increases. This is consistent with our proposed scheme. As B^z_{ext} increases, the total magnetic field experienced by the electron spin is tilted further from the (x, y)-plane, reducing the electron spin precession rate and hence the spin pumping rate. When $B^z_{ext} >> B^{xy}_{nuclei}$, the total magnetic field lies close to the z-direction and spin pumping becomes very slow, eventually becoming comparable to the hole spin relaxation rate. In this regime at ~3 T, the transmission signal is fully established. A second feature of the magnetic field dependence is the increasing contrast inequality for excitation with σ^+ relative to σ^- polarization. This dependence arises as the system moves gradually towards thermal equilibrium. The thermal population of $|\Downarrow\rangle$ (or $|\Uparrow\rangle$) increases (or decreases) with increasing Zeeman splitting and the experimental contrast for σ^+ (or σ^-) polarization tends to increase (or decrease) at high magnetic fields.

A theoretical analysis provides a quantum mechanical description of the optical pumping scheme and a means of extracting a fidelity for the hole spin initialization and the hole spin relaxation time, T^{hole}_1. The model takes a four-level basis with the coherent couplings shown in Fig. 3d. Incoherent processes, spontaneous emission, electron spin relaxation and hole spin relaxation, are included with a master equation for the density matrix. The density matrix is linked to the experiment by computing the quantum dot polarization: the differential transmission signal arises through a destructive interference of the laser and Rayleigh scattered fields. The model is explained in detail in the Supplementary Information. All the parameters for the model are known, apart from T^{hole}_1, which we determine by fitting the calculated signal strength to the experimental data (Fig. 4a).

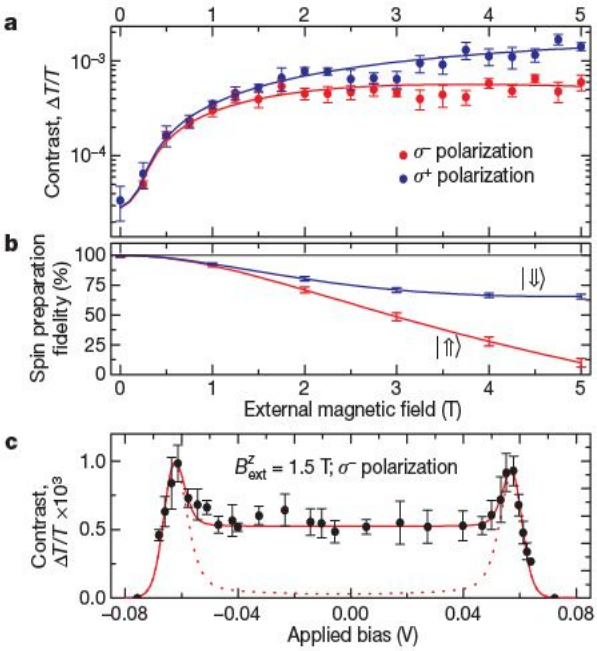

Figure 4 | Dependence of optical spin pumping on magnetic field and applied bias. **a**, Measured contrast for one-colour excitation, either σ^+ or σ^- polarization, as a function of external magnetic field applied in the **z**-direction. The solid lines represent a fit to the results of the four-level theoretical calculation using $B^{xy}_{nuclei} = 21$ mT, $E^e_{Zeeman} = 35\ \mu eV\ T^{-1}$ ($g_e = -0.66$), $E^h_{Zeeman} = 70\ \mu eV\ T^{-1}$, $\hbar\Omega_N = 0.73\ \mu eV$, $T = 4.2$ K, $T^{electron}_1 = 10$ ms, $T^{hole}_1 = 1$ ms, and $\alpha_0 = 0.025$. Only T^{hole}_1 was used as a fitting parameter. For σ^+ polarization, $\hbar\Omega_+ = 0.38\ \mu eV$, $\hbar\Omega_- = 0.02\ \mu eV$. The ratio $\frac{\Omega_+}{\Omega_-}$ represents the degree to which the polarization is controlled in the experiment. For σ^- polarization, $\hbar\Omega_+ = 0.02\ \mu eV$, $\hbar\Omega_- = 0.38\ \mu eV$. The error bars correspond to s.d. for $n = 10$. **b**, The spin preparation fidelity calculated using the fits in **a** for each hole spin state. The label identifies the spin shelving state. The error bars correspond to the range in fidelity obtained by varying T^{hole}_1 from 0.2 to 1.0 ms. **c**, Contrast versus applied bias at 1.5 T for σ^- polarization. The solid red line is the result of the four-level calculation, including both cotunnelling-related (bias-dependent) and phonon-related (bias-independent) hole spin relaxation, while the dashed red line includes only cotunnelling-related hole spin relaxation. The error bars correspond to s.d. for $n = 6$.

The theory fit to the two data sets in Fig. 4a is excellent over the full two decades of contrast and yields $T^{hole}_1 = 1$ ms for $B^{xy}_{nuclei} = 21$ mT. Values for B^{xy}_{nuclei} for similar dots vary in the literature from 12 to 26 mT (refs 7, and 10–12). Within this range of B^{xy}_{nuclei} we generate equally good fits to the data with $0.2 < T^{hole}_1 < 1$ ms, defining our uncertainty in T^{hole}_1. The T^{hole}_1 we determine is very similar to that determined from ensemble polarization decay measurements on similar quantum dots at high fields, $B^z_{ext} > 1.5$ T (ref. 23), where any hyperfine interaction is suppressed by the mismatch in hole spin and nuclear spin Zeeman energies. In our case, our experiment is most sensitive to T^{hole}_1 at low fields where the external magnetic field does not suppress the hyperfine interaction.

The theory perfectly reproduces the repumping experiment in Fig. 3d, in particular the asymmetry about the peak. The electron spin hyperfine interaction couples the two exciton states $|\Uparrow\Downarrow,\uparrow\rangle$ and $|\Uparrow\Downarrow,\downarrow\rangle$, which are diagonalized with a quantization axis tilted from z. In the tilted basis, the exciton states become $\left|\Uparrow\Downarrow,\tilde{\uparrow}\right\rangle$ and $\left|\Uparrow\Downarrow,\tilde{\downarrow}\right\rangle$, and there are two repumping transitions: at $B^z_{ext} = 0.25$ T, $|\Uparrow\rangle \leftrightarrow \left|\Uparrow\Downarrow,\tilde{\uparrow}\right\rangle$ is strong and $|\Uparrow\rangle \leftrightarrow \left|\Uparrow\Downarrow,\tilde{\downarrow}\right\rangle$ is weak. The asymmetry in the repumping spectrum arises because at large laser energies there is a small contribution from the weak repumping transition. The asymmetry therefore provides further evidence for the coupling of the two exciton states.

We define the fidelity of the hole spin initialization as $\left(\frac{\rho_{22}-\rho_{11}}{\rho_{11}+\rho_{22}}\right)$ for excitation with σ^+ polarization and as $\left(\frac{\rho_{11}-\rho_{22}}{\rho_{11}+\rho_{22}}\right)$ for σ^- polarization where ρ_{11} (or ρ_{22}) is the population of state $|\Downarrow\rangle$ (or $|\Uparrow\rangle$). The experiment is sensitive not to the populations but to the off-diagonal components of the density matrix. We use the theory to link the two. Under the experimental conditions, a slightly elliptically polarized excitation ($\sigma^+ = 99.7\%$; $\sigma^- = 0.3\%$ in power), we deduce a fidelity of $99.0 \pm 0.5\%$ at $B^z_{ext} = 0$. The uncertainty arises through the uncertainty in B^{xy}_{nuclei}. The main experimental limitation is at present the polarization: for pure σ^+ polarization, the theory predicts an increase in fidelity to above 99.9%. Figure 4b shows how the fidelity falls as the spin pumping mechanism is gradually turned off with an external magnetic field. We note that these results are not restricted to this particular dot: we have demonstrated high fidelity hole spin preparation on about ten different quantum dots.

We turn to the limiting hole spin relaxation mechanism. Cotunnelling is one possibility. A single hole is trapped in the dot over an applied bias range. At the edges of this voltage plateau, the quantum dot and Fermi energies are nearly degenerate, enabling fast hole spin relaxation through cotunnelling[6,7,27]. Consistent with this, the transmission contrast increases at the plateau edges (Fig. 4b), because cotunnelling now dominates the hole spin relaxation. We can fit the data to determine a cotunnelling spin relaxation time at the plateau edge of ~3 μs. We then predict the cotunnelling spin relaxation time at the plateau centre to be ~100 ms. This time of 100 ms is much longer than the T^{hole}_1 deduced from the data in Fig. 4a, and demonstrates that cotunnelling is not the fastest hole spin relaxation mechanism in the plateau centre. Instead, it is likely that a combination of spin–orbit and hole–phonon interactions ultimately limits the coherence of the hole spin[22,23].

The high-fidelity hole spin initialization demonstrated here opens the way to the generation of arbitrary hole spin states either by electric field induced spin resonance[29] or by an all-optical process, stimulated

Raman adiabatic passage[30]. It is tantalizing to note that in the limit in which hole spin relaxation is limited by an interaction with the phonon bath, T_2^{hole} has been predicted[4] to reach the maximum value of $2T_1^{\mathrm{hole}}$, which would correspond to a millisecond timeframe. In the context of quantum information processing, this would allow many quantum operations to be executed before the hole spin dephases.

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LETTERS

Emergent reduction of electronic state dimensionality in dense ordered Li-Be alloys

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High pressure is known to influence electronic structure and crystal packing, and can in some cases even induce compound formation between elements that do not bond under ambient conditions[1–3]. Here we present a computational study showing that high pressure fundamentally alters the reactivity of the light elements lithium (Li) and beryllium (Be), which are the first of the metals in the condensed state and immiscible under normal conditions[4,5]. We identify four stoichiometric Li_xBe_{1-x} compounds that are stable over a range of pressures, and find that the electronic density of states of one of them displays a remarkable step-like feature near the bottom of the valence band and then remains almost constant with increasing energy. These characteristics are typical of a quasi-two-dimensional electronic structure, the emergence of which in a three-dimensional environment is rather unexpected. We attribute this observation to large size differences between the ionic cores of Li and Be: as the density increases, the Li cores start to overlap and thereby expel valence electrons into quasi-two-dimensional layers characterized by delocalized free-particle-like states in the vicinity of Be ions.

Our extensive structural search exploring possible Be-Li compound formation under pressure uses two conceptually different approaches. In the first, we propose possible structures on the basis of established chemical/physical heuristics of alloy stability at 1 atmosphere (atm) and/or high pressure. Phenomenological 1-atm binary intermetallic structure maps[6] proved useful in this endeavour. In the second approach, initial structures are formed from randomly generated unit cells and atom positions, and subsequently optimized using density-functional theory (DFT)[7], with the random search targeting unit cells with 15 or fewer atoms. This combined static-lattice structure search explores the stoichiometries most common in binary intermetallic compounds, including LiBe, $LiBe_2$, Li_2Be, $LiBe_3$, Li_3B, Li_2Be_3, Li_3Be_2, $LiBe_4$ and Li_4Be.

The enthalpy of formation of Li_xBe_{1-x} is defined as $h_f(Li_xBe_{1-x}) \equiv h(Li_xBe_{1-x}) - xh(Li) - (1-x)h(Be)$, where all enthalpies h are given per atom, at the same pressure, and for temperature $T \to 0$. Thermodynamically, an alloy phase is stable against decomposition to elements if its h_f is negative. The enthalpies of elements are those of the most stable known structure of elemental Be and Li at a given pressure. Elemental Be adopts the hexagonal close-packed (h.c.p.) structure[8] at low temperature in the pressure range considered, 0–200 gigapascals (GPa), whereas Li in this pressure range undergoes a series of phase transitions: b.c.c. → f.c.c. → $cI16$ (refs 9, 10; b.c.c., body-centred cubic; f.c.c., face-centred cubic).

It is important to recognize that in these light-element phases (elements and alloys), ion dynamics can significantly change the total energies[11]. On the other hand, DFT static-lattice energies in general reproduce experimental phase stabilities well even for light-element high-pressure phases[10], in part owing to the cancellation of the dynamical effects in the energy differences. In the discussions that follow, we will use the static-lattice enthalpies to deduce the phase stabilities of Li-Be alloys under pressure, a procedure that is adequate in view of the large values of h_f we obtain. At finite temperatures, entropic contributions are potentially important. We estimate that the electronic[12] and vibrational (Supplementary Information) entropic contribution to the free energy differences between the various Li-Be phases is of the order of ~10 meV per atom at 300 K, about an order of magnitude smaller than the calculated ground state enthalpies of formation for the most stable phases.

Figure 1 shows the static-lattice enthalpies of formation per atom at selected pressures for the most competitive phase at each stoichiometry examined. Note that according to the definition of h_f and the tie line (Fig. 1 inset), a phase is thermodynamically stable if no tie line passes below it (multiple stable phases with different compositions can occur at the same pressure). Which phase will form in experimental synthesis will depend on the ratio of Li:Be loaded in the high-pressure cells, and on other experimental factors. Kinetic effects and metastability are not considered at this level.

Our calculations indicate that for all these Li-Be ordered alloy phases, the h_f values are significantly positive (>99 meV per atom, see Supplementary Information) at pressure $p = 0$, consistent with the fact that no compound or alloy forms at 1 atm. But on initial compression, the h_f values of all stoichiometries drop rapidly. At around 15 GPa, the computed h_f of the most stable $LiBe_2$ ordered alloy is already zero, and reaches a value 37 meV per atom lower than that of the separated elements at 20 GPa (Fig. 1). At the latter pressure, $LiBe_4$ and LiBe are also stable compared to the elements, but are unstable to decomposition into $LiBe_2$ and the corresponding elements, as indicated by the tie lines in Fig. 1.

Beyond 20 GPa, the h_f values of all Li-Be compounds continue to decrease rapidly with increasing pressure. At 80 GPa, the h_f of the most stable phase of each stoichiometry is substantially negative, with values for $LiBe_4$, $LiBe_2$, Li_2Be_3 and LiBe all below −100 meV per atom. The h_f of $LiBe_2$ is especially low, at −159 meV per atom. As indicated by the tie lines, at 80 GPa, $LiBe_4$, $LiBe_2$ and LiBe are stable, and are expected to be accessible to experimental synthesis in view of their substantially negative heats of formation. Above 80 GPa, the h_f values of the stable Li-Be alloys change rather slowly with pressure. At 160 GPa, we can see that only $LiBe_4$ and $LiBe_2$ are stable, and that LiBe is unstable to decomposition to $LiBe_2$ and Li.

The pressure ranges in which the four Li-Be phases are computed to be stable are depicted in Fig. 2a. Phonon calculations for $LiBe_4$, $LiBe_2$ and LiBe at 80 GPa reveal no imaginary frequencies, indicating these phases possess stable (local) minima. The most stable Li_3Be phase has a soft mode, which leads to a unit cell doubling with a stabilization of less than 2 meV per atom.

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The structures of the stable Li-Be ordered alloy phases at around 80 GPa are displayed in Fig. 2b–e. The $LiBe_4$ phase (Fig. 2b) has bilayers of puckered graphene-like sheets that sandwich Li atoms between them, with Li atoms capping the puckered hexagons. In $LiBe_2$ (Fig. 2c), alternating Be and Li graphene-like sheets sandwich Be atoms. LiBe again has layers of puckered square nets of Li alternating with layers of puckered triangular Be nets (Fig. 2d). We emphasize that we use 'layer' as a structural descriptor; for these three phases, the interatomic distances within each layer and between layers are comparable (~2 Å at this pressure). The layering, viewed in a three-dimensional context, is quite unlike, for example, graphite, which has a larger separation between layers (~3.4 Å) than within each layer (C–C bonds ~1.42 Å). Finally, we show one view of the stable monoclinic $BeLi_3$ phase (Fig. 2e); zigzag chains of Be atoms run through channels formed by the Li host.

To understand the electronic factors governing the stability of these high-pressure Li-Be alloys and the role of crystal symmetry and geometrical structure factors, we gain some guidance by simulating the expected forms of integrated X-ray diffraction patterns[11,13,14] of the four stable phases at around 80 GPa. For a range of reciprocal lattice vectors, relative peak heights can be obtained from a knowledge of the atomic scattering factors of Li, or Be, and the location of the associated basis vectors within the cell of the selected structure (Fig. 3a). Each of the four stable phases shows a manifold of strong diffraction peaks clustered around a narrow range of wavevectors and noticeably stronger (by at least an order of magnitude) than all other peaks. It is within these prominent clusters that $2k_F$ invariably lies (here k_F is the free-electron Fermi wavevector). As the diffraction intensity approximately links to the strength of a Fourier component of a self-consistent screened one-electron crystal potential in the electronic case, this observation hints at a Hume-Rothery stabilization mechanism[11,14–17]; that is, these structures enjoy relatively large electronic stabilization through the interaction between an initially free-electron Fermi surface and the Brillouin-zone planes associated with the strongest Fourier components. This can be seen, for example, from the massive pseudogap into which the Fermi level falls in the electronic density of states (DOS, or g) of LiBe at 82 GPa (Fig. 3b).

However, there is something particularly unusual about the valence electronic DOS of $P2_1/m$ LiBe at about 82 GPa (see Supplementary Information for the band structure), and of a metastable $LiBe_2$ ($Pmma$) phase at the same pressure (Fig. 3b). The structure of the $Pmma$ $LiBe_2$ (Fig. 3b inset) is closely related to the $P2_1/m$ LiBe structure, the only structural difference being the existence of two puckered triangular sheets in every Be layer in the former compound, instead of only one in the latter. This $LiBe_2$ structure does not lead to a minimum enthalpy phase (for that see Fig. 2c). It is included here as a 'tuning probe', to gauge the effect of Be layer thickness.

In both compounds the bottom of the DOS of the valence band displays a striking step-function-like singularity, with a width less than 0.1 eV. After the sharp step, the DOS remains essentially constant as energy increases but is then followed by a steep rise at about 4 eV above the bottom of the valence band, after which more distinct features emerge. The sharp step-like features in the DOS are suggestive of a van Hove singularity of a two-dimensional electronic structure, which is quite common in layered crystals (see, for example, ref. 18) but highly unusual for a compound of two metallic elements. The flat DOS places a more stringent constraint on the dispersion relation of electronic Bloch states, namely, a separable form $\varepsilon(k_x) + \varepsilon(k_y)$, where $\mathbf{k}$ is a wavevector of Bloch states in a crystal, ε is the energy of an electronic state (and is quadratic in wave-vector), and the x–y plane is the plane in which the two-dimensional character prevails. These features are especially remarkable considering the manifestly three-dimensional geometric structure of these ordered alloys.

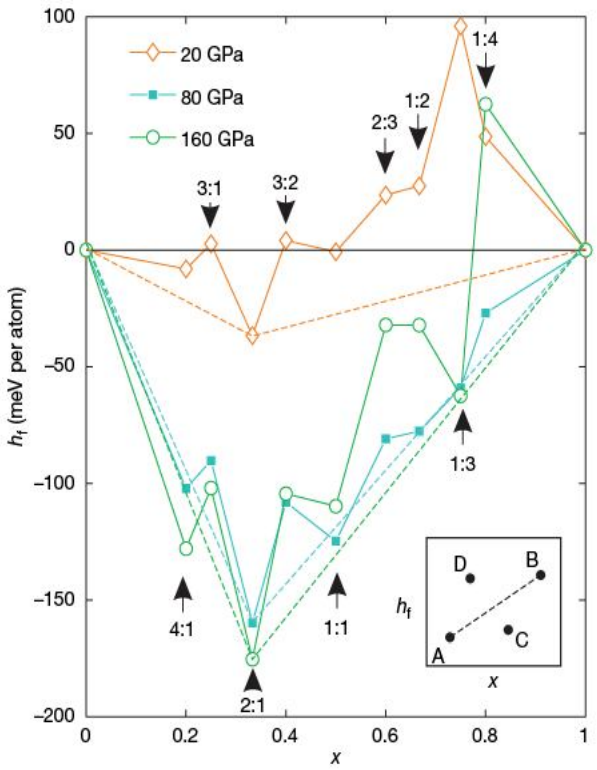

Figure 1 | Computed relative enthalpy diagram of Li_xBe_{1-x} ordered alloys. The abscissa x is the fraction of Li in the alloy, and the ordinate h_f is the enthalpy of formation per atom. Arrows indicate the Li:Be ratios. At each pressure shown, tie lines are drawn between the $LiBe_2$ phase, which has the most negative h_f, and the separated elements. Depicted schematically in the inset at lower-right is the tie line construction on a x–h_f phase diagram at a given pressure. A tie line (the dashed line) is drawn to connect phases A and B. If a third phase, such as C, lies below the tie line, it follows then from basic thermodynamic definitions that A should react with B to form C, given there is no other competing phase. Conversely, phase D, which lies above the A–B tie line, is expected thermodynamically to decompose into A and B.

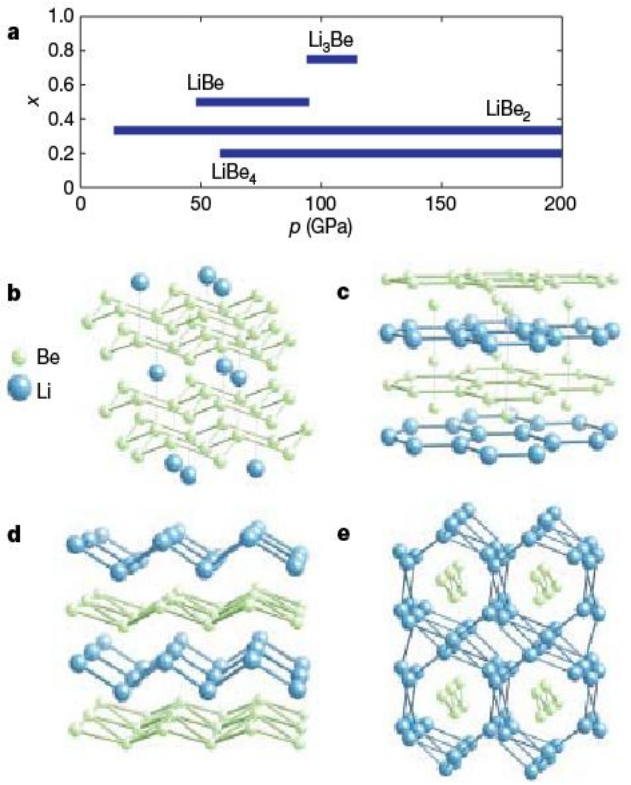

Figure 2 | Structure of stable high-pressure Li-Be compounds. Shown are Be-Li ordered alloys predicted to be stable. **a**, Stability range of the four stable Li_xBe_{1-x} alloys. **b**, $LiBe_4$ at 80 GPa (space group R-3m). **c**, $LiBe_2$ at 83 GPa ($P6/mmm$). **d**, LiBe at 82 GPa ($P2_1/m$). **e**, Li_3Be at 80 GPa ($C2/m$). Detailed structural information on these important phases is given in Supplementary Information.

To understand how such effective electronic two-dimensionality could arise in a material that by structural criteria is patently three-dimensional, we propose the following model hamiltonian in the limit of non-interacting electrons;

$$H = \sum_{k} \frac{\hbar^2 k^2}{2m^*} c_k^{\dagger} c_k + \sum_{2i} \varepsilon_o n_{2i} + \sum_{2i+1} (\varepsilon_o + W) n_{2i+1} + \sum_{i} -t\left(c_i^{\dagger} c_{i+1} + c_{i+1}^{\dagger} c_i\right) \quad (1)$$

where the first term describes the in-plane (x–y) levels of a two-dimensional free-electron gas (where the ks have no components in the z-direction), and the second and third terms embed a potential energy difference, $W > 0$, between Be and Li layers with site indices n_i (odd-numbered sites have higher energy). In equation (1), m^* is the electronic effective mass, and c and $c^{\dagger}$ are annihilation and creation operators. The last term describes the hopping perturbation between neighbouring Be and Li layers, characterized by a hopping energy, $t > 0$. The above hamiltonian can be solved exactly; in the limit $W \gg t$, it yields a DOS as shown in Fig.3b top inset, where $\tau \equiv t^2/W$.

In spite of the simplicity of this hamiltonian, it reproduces the essential feature of the lower valence DOS, computed using a DFT method that in principle includes full atomic potentials and electron–electron interactions. Moreover, in Fig. 3b left inset we see that the Be layers of the metastable $LiBe_2$ are twice as thick as in the LiBe phase, and the step in DOS of $LiBe_2$ occurs at a lower energy with roughly half the height of the LiBe case. This indicates that the two-dimensional states are associated with the Be layers. Hence, the high-potential-energy sites are associated with the Li layers, and correspondingly, the Be layers can be pictured as two-dimensional potential wells.

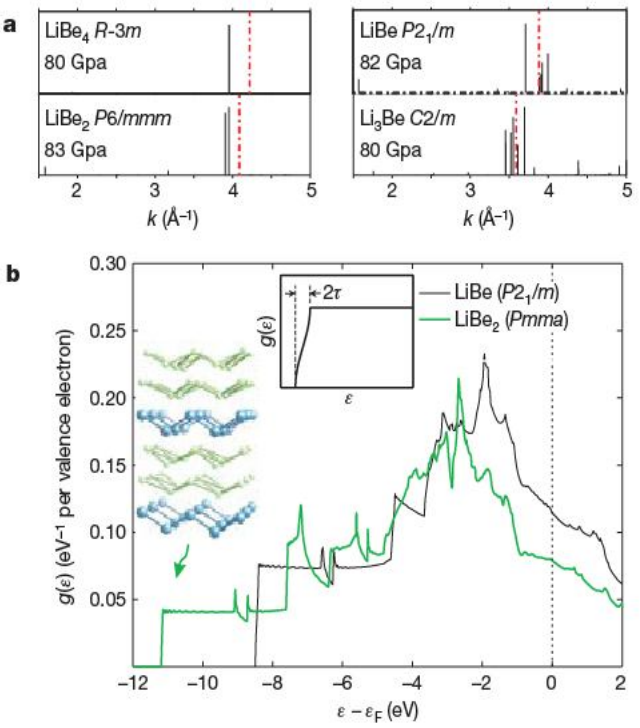

Figure 3 | Simulated X-ray diffraction patterns and electronic structures of stable $Li_{1-x}Be_x$ high-pressure phases. **a**, Approximate X-ray diffraction patterns simulated for the four stable Li-Be phases at around 82 GPa. Shown are the relative heights expected for the diffraction peaks, each associated with a reciprocal lattice vector belonging to the selected structure. The dash-dotted lines indicate where twice the free-electron Fermi wavevector lies. **b**, The electronic density of states (g or DOS) of $P2_1/m$ LiBe (82 GPa) and of the structurally related metastable *Pmma* (83 GPa) phase. Note that the DOS is given as an intensive property, that is, in units per eV per valence electron. Only the valence densities of states are shown. The dotted line indicates the Fermi level. Left inset, structure of *Pmma* $LiBe_2$. Top inset, the DOS deduced from a model hamiltonian (see text).

Figure 4 gives the computed electron density on a cross-section (6.4 Å × 16.2 Å) of the stable LiBe phase at 82 GPa. Outside the ionic core regions, a sharp separation is apparent between high-electron-density zones associated with the Be layers and low-electron-density zones associated with the Li layers. There are three density extrema outside the nuclear region: a, b and c. Extremum a is a maximum ($\sim 0.059\ e/a_o^3$, where e is the charge on an electron and a_o is the Bohr radius), from which the electron density drops slowly toward the nearest Be atom, but then very rapidly toward the nearest Li atom. Extrema b and c ($<0.015\ e/a_o^3$) are in the middle of the shortest Li–Li separations in the crystal (the Li atoms near b are above and below the plane shown). These findings are entirely consistent with the two-dimensional electron gas model described by our model hamiltonian, and confirm that the two-dimensional electron gas states are clearly associated with Be layers.

In principle, the approximate segregation of valence electrons into high- and low-density regions could arise from the potential energy difference between Li and Be layers, as characterized by W in the model hamiltonian (equation (1)). The hopping energy t for Li and Be has a typical value of ~1–2 eV (Methods). The very small value of τ (~0.05 eV) in the computed DOS indicates that W is at least ~20 eV, and hence much large than t. More importantly, a large value of W indicates that there is a very large effective electronegativity difference between Li and Be. But the first ionization potential of Be is only about 4 eV greater than that of Li, which is insufficient to produce the narrow steps at the bottom of the valence electron DOS obtained with density functional theory, and it does not explain the second step at about 4 eV above the bottom of valence bands in both *Pmma* $LiBe_2$ and $P2_1/m$ LiBe (Fig. 3b).

We suggest that the enhanced electronegativity differential arises because the potential energy difference between Be and Li increases under compression as a result of differential core overlap between Be and Li layers. Be and Li have nuclear charges of $+4e$ and $+3e$, respectively, so the 1s core of Be has a significantly smaller spatial extent than the Li 1s orbital. At 80 GPa in BeLi, both Be–Be and Li–Li bonds are between 1.9 and 2.0 Å. In fact, the electron density of a bare Li^+ at 0.95 Å away from the nucleus is 15 times that of a bare Be^{2+} (for hydrogenic wavefunctions). The difference in the spatial extent of core density is significant and even more pronounced for the common cations of these elements, whose ionic radii are 0.27 Å (Be^{2+}) and 0.76 Å (Li^+). In LiBe, the Li atomic cores have thus started to overlap significantly while the Be cores have not. Indeed, in the DFT derived band structure, the Li 1s bands show a dispersion of over 1 eV, while those of Be remain within 0.1 eV. Substantial core overlap between Li atoms[9,10,17] results in an even higher potential energy for valence electrons, which evade the Li layers and move into the neighbourhood of Be atoms where they form almost ideal two-dimensional free-electron-like states. With the enhanced electron transfer between Be and Li, our high-pressure LiBe alloys resemble

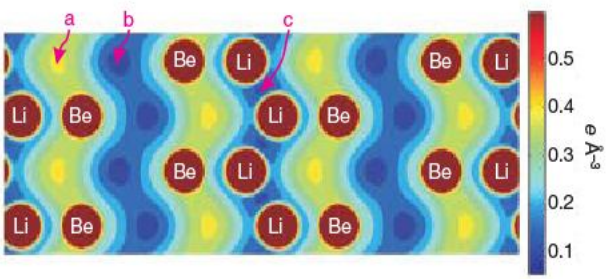

Figure 4 | Electron density of the stable LiBe phase at 82 GPa. The structure is slightly symmetrized so that we can show a plane with many atoms. The structural change in symmetrization is very small; the energy and electronic structure are essentially unchanged. Note the horizontal direction here is the direction in which Li and Be layers alternate, which corresponds to the vertical direction in Fig. 2d. a, b and c are density extrema (see text).

Zintl-type[19] compounds stabilized by electron transfer and the subsequent new bond formation.

We conclude by noting that because of its increased density of states at the Fermi energy compared particularly to elemental Be, and in view of the high dynamical energy scales of Li and Be (both superconductors in their own right), the alloy LiBe in particular may well merit assessment for superconductivity—as indeed might the other anisotropic alloys, which may even favour multi-gap pairing.

METHODS SUMMARY

We calculate the static-lattice internal energies of both Be-Li ordered alloys and the elements at given densities using the electronic DFT method[20,21] within the generalized-gradient approximation, using the Perdew-Berke-Ernzerhof exchange-correlation functional[22] as implemented in the Vienna *ab initio* Simulation Package[23]. At high density, the system is entering a regime of core overlap, and accordingly we perform all-electron calculations using a plane-wave basis set within the projector-augmented wave method[24]. For each structure, we optimize the lattice parameters and atom positions at fixed volumes, using a conjugated-gradient algorithm based on Hellmann-Feynman forces. At each volume, the structural optimization is done twice, the second starting with the optimized geometry from the first run. The optimized geometry of the various elemental phases is in good agreement with the experimental values. (For example, the equilibrium lattice constants of the b.c.c. Li and the h.c.p. Be agree with experimental values to <3%. Also, the calculated atomic volume of the *cI*16 Li at 44 GPa (8.75 Å^3) and fractional coordinates of the atom (x, x, x) with $x = 0.051$ compare very well with the powder diffraction data, 8.8 Å^3 and $x \approx 0.052$; ref. 10.) Then a single-point calculation is performed to evaluate the internal energy based on the pre-optimized geometry. The energy cut-off and **k**-point sampling are chosen such that energies are convergent within $\sim 3 \times 10^{-3}$ eV per atom over the pressure range. From calculated internal energy as a function of unit cell volume, $E(V)$, the ground state enthalpy h and hence h_f can be deduced from $h \equiv (E + pV)/N$, where N is the number of atoms, and the pressure $p \equiv -\mathrm{d}E/\mathrm{d}V$. Phonon calculations are carried out using VASP combined with PHON[25] on supercells of around 100 atoms. We use the extended Hückel method for estimating the hopping energy between Li and Be layers[26]. Symmetry identification of the random-search structures was carried out using ISOTROPY (http://stokes.byu.edu/isotropy.html).

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

Anthropogenically enhanced fluxes of water and carbon from the Mississippi River

Peter A. Raymond[1]*, Neung-Hwan Oh[1]*, R. Eugene Turner[2] & Whitney Broussard[3]

The water and dissolved inorganic carbon exported by rivers are important net fluxes that connect terrestrial and oceanic water and carbon reservoirs[1]. For most rivers, the majority of dissolved inorganic carbon is in the form of bicarbonate. The riverine bicarbonate flux originates mainly from the dissolution of rock minerals by soil water carbon dioxide, a process called chemical weathering, which controls the buffering capacity and mineral content of receiving streams and rivers[2]. Here we introduce an unprecedented high-temporal-resolution, 100-year data set from the Mississippi River and couple it with sub-watershed and precipitation data to reveal that the large increase in bicarbonate flux that has occurred over the past 50 years (ref. 3) is clearly anthropogenically driven. We show that the increase in bicarbonate and water fluxes is caused mainly by an increase in discharge from agricultural watersheds that has not been balanced by a rise in precipitation, which is also relevant to nutrient and pesticide fluxes to the Gulf of Mexico. These findings demonstrate that alterations in chemical weathering are relevant to improving contemporary biogeochemical budgets. Furthermore, land use change and management were arguably more important than changes in climate and plant CO_2 fertilization to increases in riverine water and carbon export from this large region over the past 50 years.

The riverine bicarbonate flux is a sink for atmospheric CO_2 and a small but important net flux in terrestrial systems. In the pre-industrial era, chemical weathering of silicate versus carbonate minerals sequestered CO_2 for disparate timescales owing to carbonate deposition in the oceans, with silicate weathering sequestering atmospheric CO_2 for millions of years and carbonate weathering for only tens to hundreds of thousands of years[4]. Oceanic acidification[5], however, has changed the solubility of $CaCO_3$, and considerably lengthened the timescale for CO_2 sequestration by carbonate weathering. Although the positive feedbacks between global change and chemical weathering are used in geochemical models of atmospheric CO_2 (ref. 6), these feedbacks are believed to operate on long timescales and are therefore generally left out of the current discussion on human alterations of the carbon budget. Current global carbon budgets, for example, assume that pre- and post-anthropogenic riverine carbon fluxes are equal[1].

There are two ways to increase the flux of bicarbonate from rivers. The first is to increase discharge. Although bicarbonate and most major cations/anions dilute with increasing discharge[7,8], the dilution factor in many watersheds, including the Mississippi River basin (MRB), is small, and the flux of bicarbonate increases at higher discharge[9]. The second way is to alter the relationship between discharge and carbon export, so that a higher carbon export is realized at the same discharge (Supplementary Information). Changes in this export:discharge relationship can be determined by normalizing fluxes to the rate of discharge[10,11], which we do here by calculating the bicarbonate flux at average discharge (F_{avgD}; Supplementary Information). In the Mississippi River, the bicarbonate flux and F_{avgD} show large increases from 1902 to 2005 (Fig. 1). Importantly, F_{avgD} begins to rise above the early century values around the mid-1950s (Fig. 1), resulting in a 2.9 Tg (Fig. 1 legend) larger flux during an average annual discharge year at the end of the century (Fig. 1). The 4.6 Tg (Fig. 1 legend) increase in flux lags considerably behind the increase in the F_{avgD} because there was a period of low discharge in the 1950s and 1960s (Fig. 1). Raymond and Cole[3] analysed a 1954–2001 United States Geological Survey (USGS) data set and concluded that the increase in bicarbonate flux was due mainly to an increase in discharge. This conclusion, however, was biased by a decade of relatively low flow corresponding with the start of the USGS data set. Studies that have analysed smaller temporal data sets, and not normalized for discharge, have also not found these changes in bicarbonate, because of the large annual variation in bicarbonate concentration and flux[12]. The new and higher resolution data presented here corroborate the USGS data set (Supplementary Information), yet indicate that changes in the F_{avgD} are responsible for 63% of the increase in flux (2.9 Tg of 4.6 Tg; Fig. 1 legend). This is an important distinction, which demonstrates that internal watershed modifications, discussed below, are causing a considerable change in the flux of bicarbonate and other constituents from North America's largest river.

Our sub-watershed analysis indicates that the bicarbonate flux in a large portion of the MRB is transport limited (Fig. 2). In transport limited systems, soil water ion concentrations are at or near saturation with soil minerals because of low water throughput and long water residence times[13–15]. As concentrations are at saturation and cannot be raised, this places a hydrologic constraint on the ability of additional acid generating processes (for example, soil respiration) to increase the bicarbonate F_{avgD}. There is no substantial increase in the F_{avgD} in sub-watersheds receiving less than ~0.6 m of rain, indicating severe transport limitation (Fig. 2). Watersheds receiving between 0.5 and 1 m of rain demonstrate mild transport limitation (Fig. 2). Although it is difficult to raise the rate of bicarbonate export by increasing the input of weathering agents (for example, CO_2) in transport limited watersheds, they are poised to respond to increases in discharge.

Similar to previous studies[16,17], our analysis demonstrates an increase in discharge for many gauging stations in the MRB (Fig. 3; Supplementary Fig. 4). Interestingly, by coupling watershed discharge and precipitation, we find that the discharge increases are only partly explained by concomitant precipitation increases (Fig. 3). A larger percentage of the increase in discharge, particularly in agricultural watersheds, can be explained by changes in discharge at average precipitation (Fig. 3). An increase in the discharge at average precipitation demonstrates that modifications of watershed properties are

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changing the relationship between precipitation and discharge, causing the amount of discharge to increase in a normal precipitation year (Supplementary Information). It is important to control for precipitation by calculating the discharge at the average precipitation, because the discharge:precipitation ratio naturally fluctuates with varying precipitation in a given watershed[9]. Authors have argued that whole watershed response to vegetation CO_2 fertilization should be causing increases in discharge and in the discharge:precipitation ratio by decreasing evapotranspiration[18,19]. Here we show that agricultural practices may have a stronger signal in the latter half of the twentieth century for a large river that integrates much of temperate North America. It is important to note that in forested watersheds of the MRB, increases in discharge are highly correlated with increases in precipitation (Fig. 3 legend) and not with discharge at average precipitation.

There is a strong correlation between the percentage land-cover in agriculture and the size of both the increase in discharge and the change in discharge at average precipitation (Fig. 4). We argue that the relationship between agricultural land cover and the change in discharge at average precipitation demonstrates that agricultural practices are increasing river discharge. This finding is consistent with recent studies arguing for an acceleration of the water budget of the Mississippi River due to changes in agricultural practices over

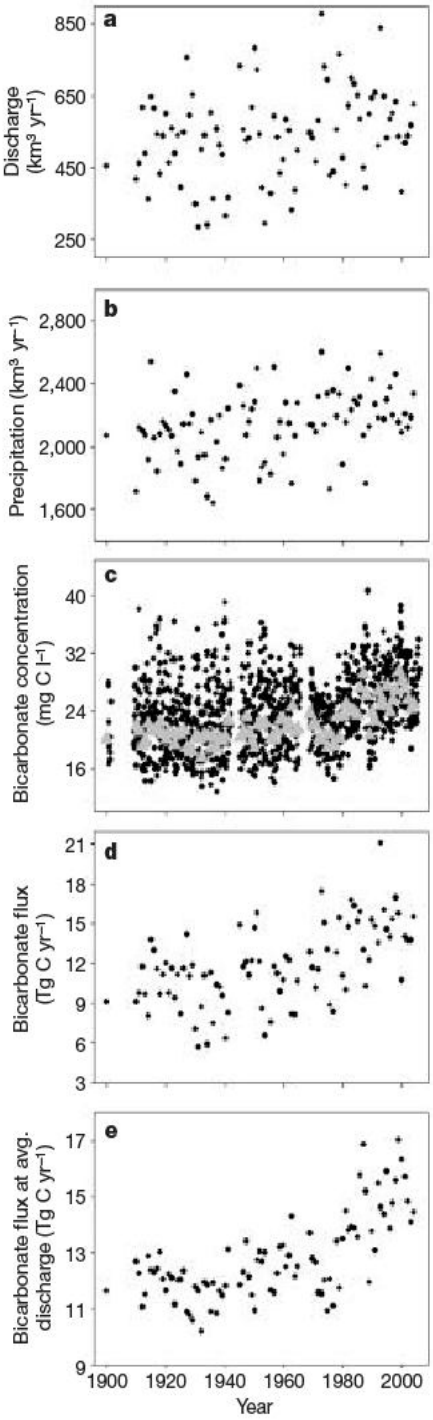

Figure 1 | Mississippi watershed fluxes and bicarbonate concentrations. **a**, Discharge; **b**, precipitation; **c**, bicarbonate concentration; **d**, bicarbonate flux; **e**, bicarbonate flux at average discharge (F_{avgD}). Concentrations represent monthly averages (black filled circles), with annual averages shown as grey filled triangles, while fluxes are all annual. The average values for pre-disturbance (<1940) are 494 km^3, 2,047 km^3, 20.3 mg l^{-1}, 10.0 Tg and 11.8 Tg for discharge, precipitation, concentration, flux and F_{avgD}, respectively. The post-disturbance (>1980) era values are 578 km^3, 2,239 km^3, 25.4 mg l^{-1}, 14.6 Tg and 14.7 Tg, resulting in changes of 84 km^3, 192 km^3, 5.1 mg l^{-1}, 4.6 Tg and 2.9 Tg, for discharge, precipitation, concentration, flux and F_{avgD}, respectively.

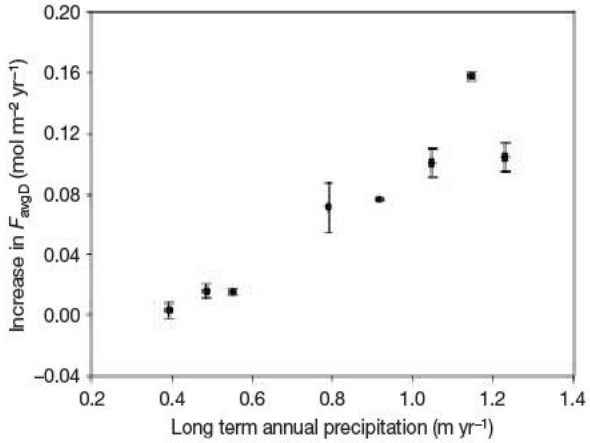

Figure 2 | Bin-averaged relationships between the increase in F_{avgD} and annual precipitation for sub-watersheds of the Mississippi. Non-responses from watersheds with low (<0.6 m yr^{-1}) levels of precipitation are an indication of transport limitation. Only watersheds that had an increase in F_{avgD} were used. The error bars are ±1 s.e. ($n = 6$ for each bin). The precipitation bins are 0.33–0.42 cm, 0.45–0.53 cm, 0.54–0.57 cm, 0.58–0.90 cm, 0.91–0.93 cm, 0.94–1.12 cm, 1.12–1.17 cm and 1.19–1.36 cm.

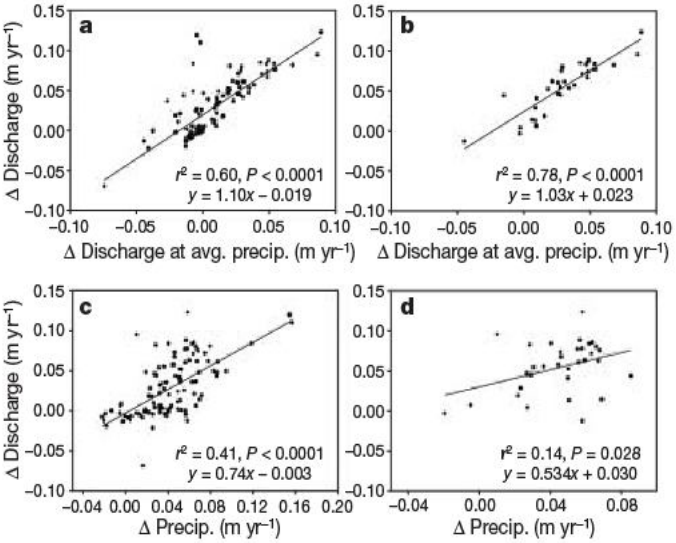

Figure 3 | Change in discharge versus change in discharge at average precipitation and change in precipitation for sub-watersheds of the Mississippi. The left panels show all watersheds, while the right panels show only watersheds with 70% or greater cropland land cover. The change was calculated by grouping the data before 1966 and after 1987. It is important to note that in sites with low agricultural cover (<30%), change in precipitation predicts 74% of the variation in change in discharge, while change in discharge at average precipitation can only predict 25%—that is, in forested watersheds changes in precipitation do balance changes in discharge.

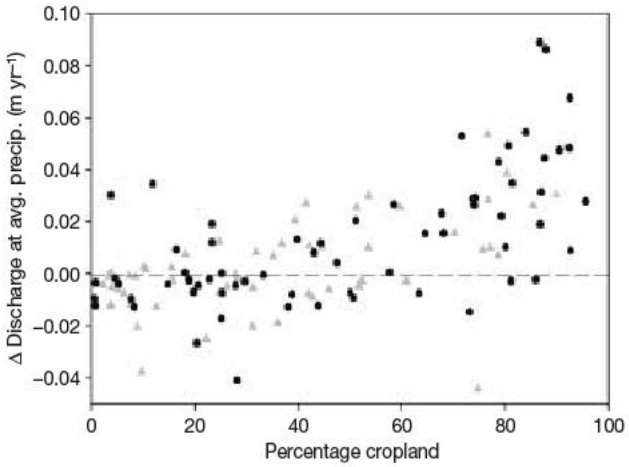

Figure 4 | The change in discharge at average precipitation versus the percentage of land-cover designated as cropland for sub-watersheds of the Mississippi. The change was calculated by averaging the time periods <1966 and >1987. The black filled circles represent watersheds that are independent, while the grey filled triangles are watersheds that have nested watersheds within them.

similar time periods[20,21]. Remembering that these agricultural landscapes are highly modified, have soils that are well buffered and moderately transport limited, and therefore have high concentrations of bicarbonate and other important biogeochemical constituents[22], this amounts to significant increases in fluxes from the US cropland centres. Agricultural liming is undoubtedly playing a role in the increased bicarbonate fluxes[23–25], although the impact of liming will be hydrologically constrained in transport limited watersheds.

We can use the data presented here to scale the potential ramifications of increased discharge, due to agricultural practices, on water and bicarbonate fluxes. If one assumes that 42% of the Mississippi watershed is cropland (Supplementary Information) and that there is on average a 4 cm (Fig. 4) increase in discharge not balanced by precipitation on this land-cover, then the annual increase in discharge not balanced by precipitation is 49 km^3. If we assume that average bicarbonate concentrations from croplands are ~50 mg Cl^{-1} (ref. 3), the result is an additional 2.4 Tg yr^{-1} of bicarbonate, or about 52% of the increase in flux found in this study. Owing to the much higher bicarbonate yields from these well buffered agricultural soils, increasing the proportion of water originating from croplands will account for 84% of the increase in the F_{avgD} (Fig. 1). The high concentrations of bicarbonate (50 mg Cl^{-1}) in cropland rivers and streams are also partly due to agricultural practices like liming[23–25], and therefore agricultural practices that have raised bicarbonate concentrations are also partly captured in these estimates. Precipitation has increased by 190 km^3 (~9%) over this same time period (Fig. 1), which is consistent with estimated values[26]. Using a pre-disturbance discharge:precipitation ratio of ~0.24 (Fig. 1) and a pre-disturbance Mississippi River bicarbonate concentration of 20.3 mg Cl^{-1} (Fig. 1) results in an increase in flux of 0.94 Tg due to precipitation increases, which is ~20% of the observed increase in flux. Thus, in the MRB, despite a 9% increase in precipitation, the anthropogenic forcing has outweighed the climatic forcing in the past 50 years. The remaining 30% of the increase in bicarbonate flux is due to other mechanisms, such as recovery from acidification[27], and anthropogenic alterations that allow for a greater response to precipitation post-disturbance.

We argue that changes in agricultural practices have had drastic impacts on the net chemistry and hydrology of the Mississippi. With respect to the discharge increase, agricultural practices have led to an increase of discharge on the scale of ~50 km^3 yr^{-1} (for comparison, the discharge of the Susquehanna and Rhone rivers are ~30 and 54 km^3 yr^{-1}, respectively). Potential agricultural practices that might be altering discharge are tile drainage, fertilizer use, irrigation, tillage practices, and changes in crop type[20,21], rotation and productivity. These changes, coupled with precipitation increases and the use of lime, have led to a large increase in alkalinity flux. Large scale changes to crop production to meet proposed ethanol production may continue to alter cropland water and carbon export. Furthermore, bicarbonate is the major anion in streams and rivers and is linked to many other element cycles, so large scale changes in bicarbonate demand compensating changes in other important ions, such as calcium and silica[28]. Finally, any element (for example, nitrogen or phosphorus)[22,29] or pollutant[22,29] that has elevated fluxes due to agricultural practices is undoubtedly also being affected by the increase in discharge from agricultural watersheds, potentially on a similar scale to those reported here for bicarbonate.

METHODS SUMMARY

The data set used here for the Mississippi River represents tens of thousands of measurements over 100 years, and is therefore the most complete long-term data set for riverine carbon for a major world river ever reported and a data set of primary importance with respect to understanding global change issues. The data originate from the Carrollton and Algiers Water Purification Plants, operated by the Sewage and Water Board of New Orleans, where alkalinity is measured to determine how much lime to add as Mississippi River water is processed for the city. The plants have made multiple measurements of bicarbonate, as alkalinity (Supplementary Information), per day for the past 40 years, and perhaps the past 105 years, although only the monthly averages are available from 1902 to 1961. See Supplementary Information for further discussion of the raw data. The second data set was 106 USGS sub-watersheds that had long-term records of discharge, 84 USGS sites with long-term river chemistry data (augmented with historic data from the state of Illinois water survey to increase the number of agricultural sites), and precipitation data from the PRISM Group, Oregon State University. Explanations and examples of how fluxes were calculated are provided in Supplementary Information.

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LETTERS

Deformation and rupture of the oceanic crust may control growth of Hawaiian volcanoes

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Hawaiian volcanoes are formed by the eruption of large quantities of basaltic magma related to hot-spot activity below the Pacific Plate[1,2]. Despite the apparent simplicity of the parent process—emission of magma onto the oceanic crust—the resulting edifices display some topographic complexity[3–5]. Certain features, such as rift zones and large flank slides, are common to all Hawaiian volcanoes, indicating similarities in their genesis; however, the underlying mechanism controlling this process remains unknown[6,7]. Here we use seismological investigations and finite-element mechanical modelling to show that the load exerted by large Hawaiian volcanoes can be sufficient to rupture the oceanic crust. This intense deformation, combined with the accelerated subsidence of the oceanic crust and the weakness of the volcanic edifice/oceanic crust interface, may control the surface morphology of Hawaiian volcanoes, especially the existence of their giant flank instabilities[8–10]. Further studies are needed to determine whether such processes occur in other active intraplate volcanoes.

To understand the morphology of Hawaiian volcanoes, we must combine geophysical information about the deep structure of the volcanoes with studies of the mechanical interactions that take place within the edifices and within the oceanic crust. In this work we first computed a three-dimensional (3D) P-wave velocity model of Mauna Loa volcano and Kilauea volcano, using data from 1,435 earthquakes that were well recorded (at least 25 stations) by the Hawaiian Volcano Observatory (HVO) network between 1988 and 1999. These events were declustered in such a way that the earthquake-station ray set optimally sampled the tomographic volume. The 44,504 high-quality P arrival-times were inverted using Monteiller's probabilistic algorithm[11]. The resulting 3D velocity model (959,077 1-km^3 cubic cells) was stable and detailed, and showed most of the volcanoes' structural features (Fig. 1). Therefore, we used this model to compute double-difference locations for 1,161 highly correlated earthquakes (providing 8,444,395 cross-spectral time delays of millisecond-accuracy, from 669,903 event pairs) in the south flank of Kilauea volcano recorded between 1988 and 1999 by the HVO seismic network (Fig. 2a). The quality of the velocity model ensured that the slowness vectors used in the double-difference locations were correctly computed. Plots of time delays as a function of the angle between the slowness vector and the relative position vector for each coherent earthquake pair and each station (Supplementary Fig. 1) showed that the locations were almost perfectly coherent with the slowness vectors computed using the tomographic model. A systematic computation of the location root mean square for varying dips showed that dip is well constrained in our 3D velocity model (Supplementary Fig. 2). The double-difference location results showed earthquake locations on a subhorizontal décollement plane, and along a deeper, steeper, southward-dipping reverse fault (Fig. 2a; Supplementary Fig. 3), thus confirming some preliminary results[12,13] obtained using a one-dimensional model. The inferred depth for the décollement plane (8–9 km) was obtained by double-difference relocation using time-delay measurements computed from widely correlated events into the 3D velocity model. Coherence remained greater than 80% for inter-event distances up to 1 km, leading to a well-conditioned inversion. This provides an accuracy of better than 500 m for the earthquake depth calculations. The 8.5-km depth coincides locally with the top of the oceanic crust[14,15].

Travel-time tomography was used to determine the deep structure of the volcanoes. The resulting cross-sections show roughly triangular, high-velocity cores beneath the calderas and rifts. Plots of P-wave velocities in the south flank of Kilauea along a north–south horizontal profile (Fig. 2b) showed that velocity falls substantially with distance from the rift axis. The norm of the velocity gradient is largest near the intersection of the reverse fault plane and the décollement plane. This maximum is reached for a P-wave velocity of roughly 6 km s^{-1}, which is the limit used[14] to differentiate between intrusive rocks and lava flows in Hawaiian volcanoes. Reverse faulting initiates

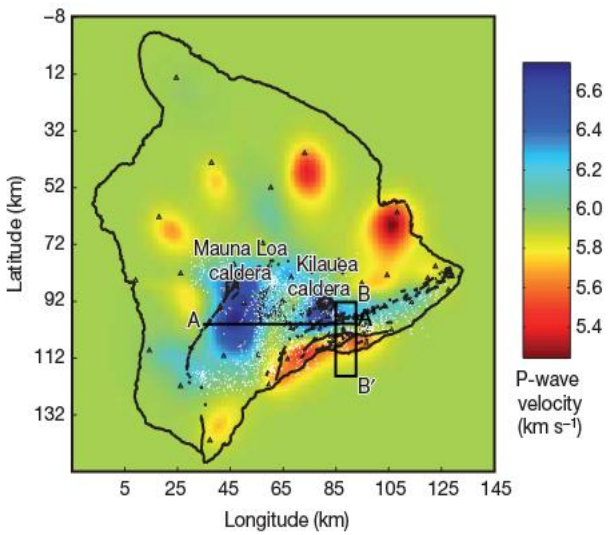

Figure 1 | Map of Hawaii Island. The map shows the main topographic features, the epicentres of the earthquakes used for the tomographic model (white dots) and for the double-difference relocation (black dots), and the location of the HVO short-period seismic stations (open triangles). The solid rectangle and the solid line show the locations of the cross-sections presented respectively in Figs 2a and b, and in Fig. 4. Colour is used to represent P-wave seismic velocities after travel-time tomographic inversion, at 6 km depth. The resulting model is the simplest that best fits the data (optimal *a priori* information: velocity parameter correlation length $\lambda = 5$ km, velocity standard deviation $\sigma_v = 1$ km s^{-1}). It may be compared to former P-wave velocity models[26,27].

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near the core/cover boundary, at the depth of the décollement plane, and propagates through the oceanic crust.

To ascertain the conditions required for such a rupture of the oceanic crust to take place, we drew up a mechanical model of the interaction between Hawaiian-type volcanoes and oceanic crust. Geometrical boundary conditions and constitutive laws of these bodies are provided by tomographic results and by Hill and Zucca's correspondence[14] between local rock formations and velocities. Drilling experiments[16] in and around rift zones have shown that the peripheral cover surrounding rift zones is mainly composed of altered lavas whose mechanical properties are characterized by low Young's modulus, cohesion and friction coefficient. We therefore represented the slow peripheral cover by an elastic-plastic material, and carried out various numerical experiments using an elastic or elastic-plastic material for the dense and fast cores (Fig. 3; Supplementary Figs 4–9). The oceanic crust was represented by an elastic-plastic material[17]. Basal décollement (top of the oceanic crust) is represented by an interface with a Coulomb friction law (angle of friction $\phi = 10°$)[4,18]. Finite-element calculations of the deformation of this model were carried out using the ADELI code[19]. They showed

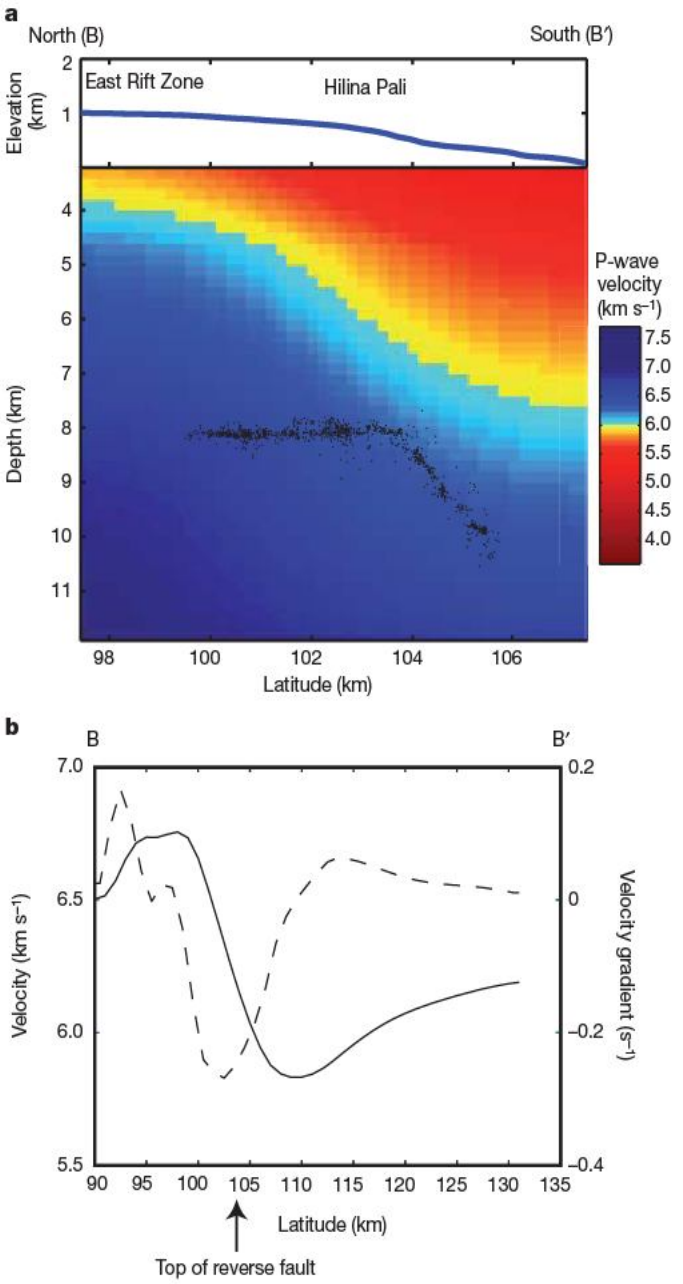

Figure 2 | Double-difference location and tomography results. **a**, North–south cross-section of the velocity model (Fig. 1) and location of relocated earthquakes on the south flank of Kilauea volcano. The colour scale highlights the velocity interval of 5.8–6.2 km s^{-1} that marks[14] the separation between intrusive rocks (core rocks) and lava flows (cover rocks). Double-difference relocation uses this tomographic velocity model, thereby ensuring that ray parameters are correctly calculated. We carried out relocation using 1,161 correlated earthquakes (coherence >80%) on the south flank of Kilauea volcano recorded by the HVO network between 1988 and 1999. The average errors for the relocations were found to be 100 m vertically and 50 m horizontally. **b**, Velocity (solid line) and velocity gradient (dashed line) as a function of distance along a horizontal north–south profile across the south flank of Kilauea. The velocity profile is the mean, at 8.5 km depth, of 10 north–south contiguous profiles across the rectangular area B–B′ indicated in Fig. 1. The arrow indicates the abscissa of the intersection of the reverse fault with the décollement plane; it shows that the reverse fault initiates in the neighbourhood of the highest velocity gradient (core/cover boundary).

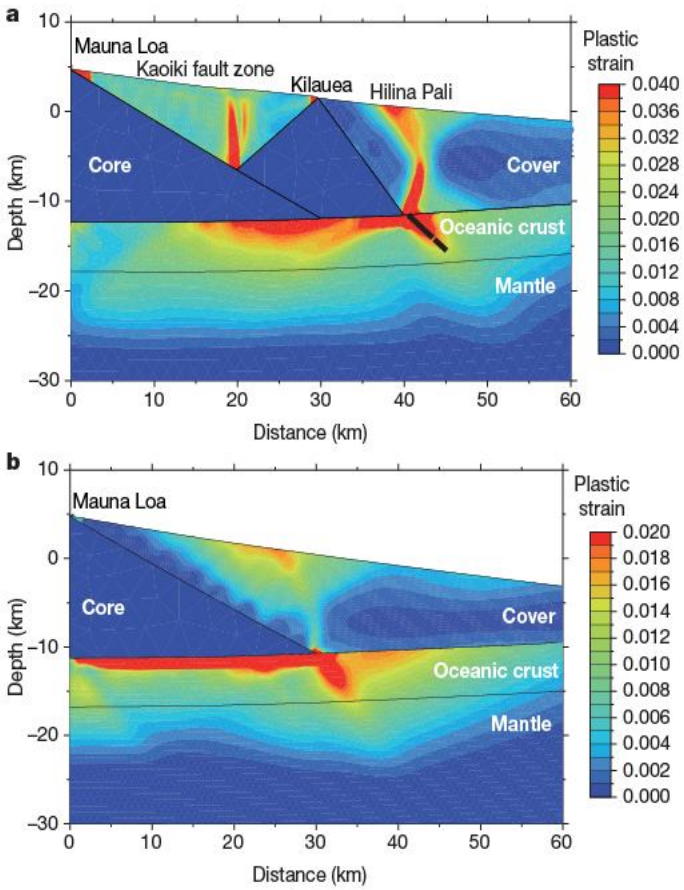

Figure 3 | Model of the mechanical interaction between the volcanic edifice and the oceanic crust. **a**, Mauna Loa and Kilauea volcanoes are both represented by a dense and elastic core (Young's modulus $E = 100$ GPa, Poisson's ratio $\nu = 0.25$, density $\rho = 2{,}900$ kg m^{-3}), and a light elastic-plastic cover ($E = 60$ GPa, $\nu = 0.25$, cohesion $C = 1$ MPa, angle of friction $\phi = 15°$, $\rho = 2{,}600$ kg m^{-3}). The oceanic crust is represented by an elastic-plastic material[17] ($E = 100$ GPa, $\nu = 0.25$, $C = 1$ MPa, $\phi = 15°$, $\rho = 2{,}900$ kg m^{-3}). The décollement plane is represented by an interface with a Coulomb friction law ($\phi = 10°$)[4,18]. The dimensions of the cores were deduced from the tomographic model. Colours are used to show the plastic deformation (norm of the deviatoric plastic strain tensor). Notice the plasticized shear zone created at the edge of the dense core. Deformation in the volcanic edifice and along the décollement plane is due to the gravitational and flexural stresses. Introducing a moderate amount of plasticity (Supplementary Fig. 4: $C = 5$ MPa, $\phi = 25°$) in the cores does not notably change the computed deformations. A homogeneous elastic-plastic model for cores, covers and oceanic crust (Supplementary Fig. 5) preserves a recognizable plasticized shear zone at the edge of the Kilauea dense core. **b**, Model of the squeezing of the elastic-plastic oceanic crust by an isolated volcano the size of Mauna Loa. A plasticized shear zone, which cuts through the entire oceanic crust, is created at the edge of the dense core. It has a dip comparable to the reverse fault dip found by double-difference relocation (Fig. 2a). Additional horizontal displacement due to the expansion of the rift zones does not change the major features evidenced at lower strain rates (Supplementary Fig. 6).

that, provided there is a realistic density contrast between the core and the cover, the deviatoric stress can locally attain the plasticity threshold and reverse faults can cut through the oceanic crust. Thus, the load of the volcano can cause intense plastic deformation of the oceanic crust, even though the volcano may be of limited size (for example, Kilauea) (Fig. 3a). A large enough volcano (for example, Mauna Loa) may rupture the oceanic crust completely (Fig. 3b). Petrological and volcanological features[20,21] have provided further evidence for oceanic crust rupture under the load of Hawaiian volcanoes. Our model also shows that the effusive cover, both between Mauna Loa and Kilauea (Kaoiki fault zone), and on the south flank of Kilauea volcano (Hilina fault scarps), is plasticized by the squeezing action of the cores of the volcanoes (Fig. 3a; Supplementary Fig. 4).

Double-difference relocation of 732 earthquakes (Fig. 4) indicated that décollement earthquakes occur at depths of around 10 to 11 km under Mauna Loa and at depths of around 8 to 9 km under the south flank of Kilauea. The vertical uncertainty for these relocations was less than 500 m. The difference in décollement depth is about 2 ± 1 km for 10 to 15 km horizontally between Mauna Loa and Kilauea volcanoes. Based on the hypothesis that the décollement plane coincides with the top of the oceanic crust, the top of the oceanic crust between Kilauea and Mauna Loa slopes at an angle of $9 \pm 5°$, whereas the average slope below the oceanic crust of the Hawaiian islands is between 1.5 and 3°. This has been confirmed by careful inspection of active seismic results[14,15]. Such local variations in flexure may be considered anomalous; they cannot be explained using homogeneous elastic models[22]. Elastic-plastic models (Fig. 3) also place the bases of Mauna Loa and Kilauea volcanoes at similar levels. If such a slope variation was confined to the 5- to 6-km-thick crust, it would represent an unrealistic deformation greater than 15%; hence this deformation must also concern the upper mantle. Interestingly, the southwest rift zone occupies the same position with respect to Mauna Loa as the reverse fault revealed by double-difference location occupies with respect to Kilauea. These observations and the results of elastic-plastic modelling on the scale of Mauna Loa (Fig. 3b) suggest that the rift system forming Kilauea may have initiated in the local rupture of the oceanic crust caused by the loading exerted by Mauna Loa, leading to magmatic eruption, collapse of the oceanic crust below Mauna Loa (eventually accelerated by the relative displacement of the mantle plume) and further propagation of the rift as fast as the volcanic load increases. At depth, the seismicity (~30 km) in the neighbourhood of the Mauna Loa/Kilauea volcano feeding system indicates movements[6] along subhorizontal planes, consistent with the flexural stresses[7] induced by the volcanic load.

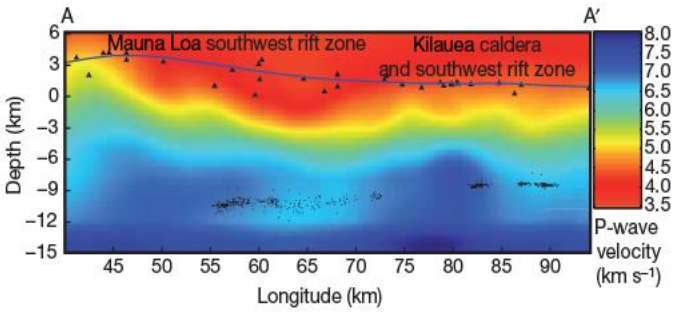

Figure 4 | East–west cross-section between Mauna Loa and the south flank of Kilauea. See earthquake locations and profile A–A′, Fig. 1. The cross-section shows the tomographic model and relocated earthquakes (black dots). Resolution of the tomographic model is represented through a chequerboard test (Supplementary Fig. 9). We identified 732 relocated earthquakes along the décollement plane beneath Mauna Loa and the south flank of Kilauea by using a cross-spectral coherence criterion (similarity of the waveforms), and relocated them using cross-spectral time delays. We selected events located beneath the easternmost part of the southeast flank of Mauna Loa (longitude: 62 to 72 km) for the similarity of their travel times and focal mechanisms, and relocated them using travel-time differences. Relocation results may be compared to former locations computed along similar profiles[28,29].

Geodetic measurements clearly show that Kilauea and, to a lesser extent, Mauna Loa grow horizontally[23–25]. Therefore, topographic variations in the top of the oceanic crust may also constrain the (horizontal) growth of Hawaiian volcanoes. It is often thought that Kilauea buttresses Mauna Loa, blocking its seaward displacement. The topography of the oceanic crust may provide a more efficient explanation. The horizontal force, F, required to move a non-deformable wedge of weight P, along a slope inclined at an angle θ, and with an internal angle of friction ϕ is $F = P\tan(\theta + \phi)$. The apparent basal friction coefficient along the décollement plane of Kilauea volcano is thought to be very low[4,18] (close to 0.1, $\phi \approx 5°$ to 10°). With such friction coefficients, slope variation appears to be a critical factor in determining the mechanical evolution of Mauna Loa and Kilauea: more than three times as much energy is required to move a body up a 10° slope than along a horizontal surface. To reach an equivalent value by increasing the weight, P, of the wedge, the thickness of Kilauea volcano would have to be double the thickness of the southeast flank of Mauna Loa, which is contrary to the best recent estimates[5].

Topographical variations in the top of the oceanic crust could also explain why the surface topographic slopes are steeper near large eruptive centres, such as Mauna Loa[3]. The apparent coefficient of friction increases with the slope of the oceanic crust and the edifice remains stable for higher topographic slopes, as long as it has sufficient internal strength[4,18]. The variations in the slope of the oceanic crust across and along the rift zones, added to the squeezing action of the central core on the plastic cover (Supplementary Fig. 6), may therefore explain the giant gravitational readjustments observed[8–10].

The topography of the oceanic crust may also be responsible for (1) the blocking and relatively weak eruptive activity along the southwest rift zone, and (2) the existence of an 'aseismic' zone along the ramp, whereas the southeast flank of Mauna Loa shows some horizontal displacement[25]. Could this zone accumulate sufficient stress to be the source of major earthquakes, such as the great Kau (magnitude ~8) earthquake, today's expression of Lipman's 'piggyback' tectonics[5]?

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LETTERS

Clusters of ant colonies and robust criticality in a tropical agroecosystem

John Vandermeer[1,2], Ivette Perfecto[2] & Stacy M. Philpott[3]

Although sometimes difficult to measure at large scales, spatial pattern is important in natural biological spaces as a determinant of key ecological properties such as species diversity, stability, resiliency and others[1–6]. Here we demonstrate, at a large spatial scale, that a common species of tropical arboreal ant forms clusters of nests through a combination of local satellite colony formation and density-dependent control by natural enemies, mainly a parasitic fly. Cluster sizes fall off as a power law consistent with a so-called robust critical state[7]. This endogenous cluster formation at a critical state is a unique example of an insect population forming a non-random pattern at a large spatial scale. Furthermore, because the species is a keystone of a larger network that contributes to the ecosystem function of pest control, this is an example of how spatial dynamics at a large scale can affect ecosystem service at a local level.

It has been common to assume, sometimes only implicitly, that the patchiness of an ecosystem reflects some underlying habitat factor (for example, marshweed occurs in marshes) even though that factor may not be evident[8,9]. However, it is well known that various intrinsic biological dynamics are capable of producing pattern even in a landscape that is homogeneous for the organism involved[10,11]. This raises the question for any non-random spatial pattern about whether it is caused by factors that are exogenous (broadly, underlying habitat patchiness) or endogenous (broadly, biological aspects of the organism independent of the habitat patchiness)[12,13].

In our study site in southern Mexico the underlying habitat for a species of tropical arboreal ant, *Azteca instabilis*, is essentially uniform. It is the collection of shade trees planted in a coffee plantation, where the original intention of the farmer was to plant the trees uniformly. The ant species is common in the Mesoamerican tropics, where it is frequently encountered on casual walks in the forest. However, discerning any spatial pattern of its colonies is inevitably obstructed by the heterogeneity of the habitat it normally occupies. However, the species also inhabits shade trees in traditional shaded coffee farms, a uniform habitat both by intention and as measured. Ant nest formation is exclusively in the shade trees; consequently a non-random pattern in the spatial distribution of ant colonies must be a result of endogenous factors, because the underlying habitat is uniform. We find that the colonies indeed are non-random, even though the shade trees themselves are uniform (Fig. 1).

The basic biology of the ant is not unusual. After a queen establishes a colony in a tree, the colony may grow to the point that satellite nests are established in neighbouring trees, presumably one part of the mechanism whereby patchiness is generated. Although the details of satellite formation are not completely known (see Supplementary Information), it is evident from our data that ants within a particular nest establish other nests in nearby trees. Unabated satellite formation would obviously result in a continuous expansion of nests throughout all shade trees in the habitat, which means that some force must limit this expansion. On this farm the ant has a series of natural enemies, any one of which, or any combination thereof, could form the basis for the control that must occur. A parasitoid phorid fly is known to reduce ant foraging activity[14], and has a

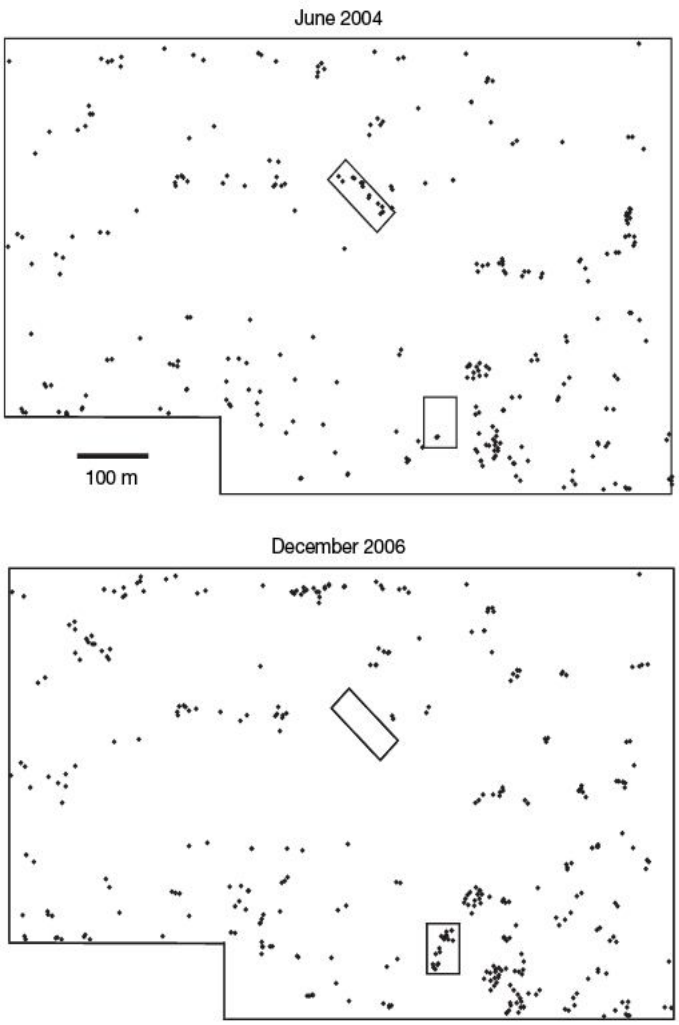

Figure 1 | Distribution of nests of *Azteca instabilis* over a 45-ha plot (the three missing hectares are on inaccessible terrain). The distributions of colonies found in all censuses are notably clumped (the first and last censuses are shown here) with an average of 328 trees occupied. The nests appear to drift around, as is evident from a comparison of the two panels. Note the dynamic nature of the system over time: the two small rectangles illustrate both the complete disappearance of a cluster and the appearance of another cluster where only a single nest had been before.

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density-dependent response to clusters of ant nests (Fig. 2), in addition to a qualitative behavioural response to the ants (see Supplementary Information).

Based on this natural history, we propose a three part dynamic. First, nuptial flights produce founding queens that disperse as propagule rain over a large area. Second, successful colonies occupy neighbouring trees with satellite colonies. Third, phorid parasitoids concentrate on clusters of ant nests, causing a dramatic behavioural response and possibly direct mortality, thus dramatically reducing ant survivorship in dense clusters of nests. The cellular automata model developed here (see Methods and Supplementary Information) is based on these three features, where a central cell becomes occupied or dies depending on the Moore neighbourhood, N, with the probability of satellite expansion being a linear function of N ($p_s = s_0 + s_1 N$), as is the probability of mortality ($p_m = m_0 + m_1 N$).

The range of parameter values to instantiate the cellular automata model (see Methods and Supplementary Information) obtained from the field censuses were: for satellite expansion, $s_0 = 0.0$–0.8, $s_1 = 0.0133$–0.035; and for mortality, $m_0 = 0$–0.45, $m_1 = 0.031$–0.097. A systematic search of this range of parameter space produced the following parameters: $s_0 = 0.0035$, $s_1 = 0.035$, $m_0 = 0.116$, $m_1 = 0.036$, as those producing the best approximation to both the population densities of nests over time and the cluster size distribution (as measured by the mean/variance ratio). Output from the model and observed data from the field are shown in Fig. 3.

The overall population densities in the simulations are concentrated between 200 and 500, and the mean variance ratios between 0.4 and 0.5, both close to the range of our observations in nature (represented as horizontal lines in Fig. 3). The model output reflects the erratic nature of cellular automata models, with the same parameters generating a dramatic variability both of population densities and mean variance ratios. However, because the possible range could be from 0 to 10,800 for population density and from 0 to +infinity for mean variance ratios, the ability of the model, with parameter values within our empirical envelope, to generate population densities and patterns so close to those we observed in the field suggests that the basic interpretation of the spatial dynamics is probably correct.

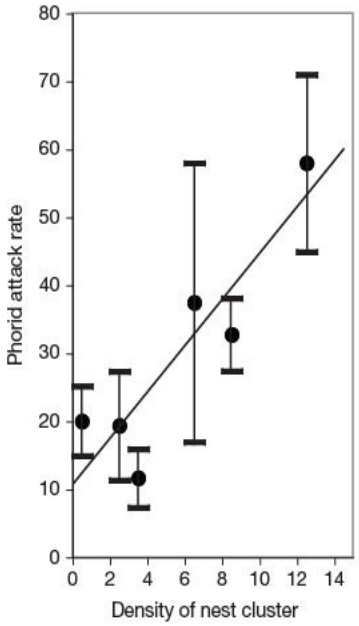

Figure 2 | Attack rates of phorid fly parasites as a function of nest-cluster density. Density of nests based on a 20 m circle surrounding the point at which phorid attack trials were done. Error bars, s.e.m. The attacks were highly variable, but the relation with the density of the local clusters of ant nests is statistically significant ($P = 0.042$; see also Supplementary Information).

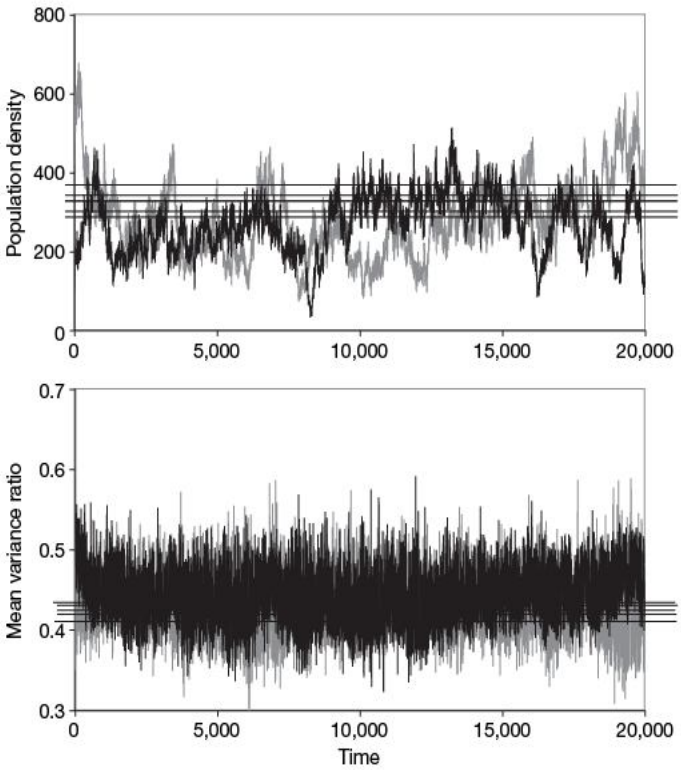

Figure 3 | Time (in six month intervals) series for population density (top) and mean variance ratios (bottom) for the parameters $s_0 = 0.0035$, $s_1 = 0.035$, $m_0 = 0.116$ and $m_1 = 0.036$. In both cases two separate runs are pictured: one in black, the other in grey (hardly noticeable in the bottom panel because the two runs are so similar). Horizontal lines are the values of the six field samples (two samples are so close as to appear the same).

Although our modelling approach is distinct, the underlying biological interactions are similar to those studied by Pascual and colleagues, suggesting that we should expect a power law relation between cluster size and frequency. Indeed, as expected, the distribution of cluster sizes in our plot does follow a power function (Fig. 4a). Furthermore, calculating the frequency of cluster sizes, as generated by the model, produces a similar power relation (Fig. 4b) as would be suggested if the system is near criticality[15]. Further studies of the model show that there is a broad region of parameter space in which the power law holds, suggesting that this may be a case of robust criticality[16].

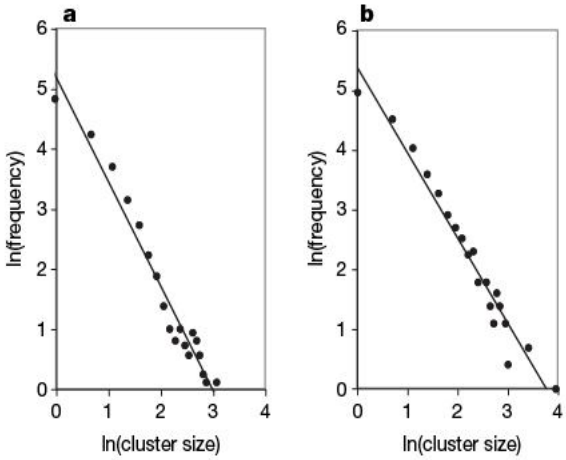

Figure 4 | Log of cumulative frequency of log cluster sizes. a, From field samples, based on a minimum distance of 20 m between nests that are judged to be in the same cluster. **b**, From field-parameterized cellular automata stochastic model, based on a 90 × 120 lattice, where each lattice point is intended to model a single shade tree. Clusters are defined based on individual lattice points in contact with any other lattice point in the Moore neighbourhood.

The importance of these results lies in the fact that strong spatial pattern is formed in the face of habitat homogeneity, connecting with the well-known consequences of spatial pattern on topics such as species diversity, ecosystem stability or resiliency and others. Furthermore, it is likely that this pattern formation also is related to biological control of several important coffee pests such as the coffee berry borer (*Hypthenemus hampei*)[17], the green coffee scale (*Coccus viridis*)[18] and coffee rust (*Hemileia vastatrix*)[19]. All of these controls are effected through the spatial patterning of this system, as discussed in detail elsewhere[20].

METHODS SUMMARY

All trees in a 45-ha plot in a shaded coffee plantation were located and the presence of ant colonies therein noted, a process repeated at 6-month intervals for 2 years. Non-randomness of nests was established through quadrat-based computation of mean and variance of number of nests per quadrat. Attack rates of phorid flies were determined in locations that varied in the local number of ant nests, by placing a small amount of ant-nest carton with a few ants in a container and counting the number of phorid attacks over a 20 min period. The stochastic cellular automata was constructed based on the natural history observations of the ants forming satellite nests on nearby trees plus the phorid flies encountering nests in proportion to their local density. The model was parameterized with field data and compared with actual distributions in the field. Both the distribution of nests in the field and the distribution generated by the cellular automata were examined for the distribution of cluster sizes.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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LETTERS

Histone H2AX-dependent $GABA_A$ receptor regulation of stem cell proliferation

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Stem cell self-renewal implies proliferation under continued maintenance of multipotency. Small changes in numbers of stem cells may lead to large differences in differentiated cell numbers, resulting in significant physiological consequences. Proliferation is typically regulated in the G1 phase, which is associated with differentiation and cell cycle arrest[1]. However, embryonic stem (ES) cells may lack a G1 checkpoint[2,3]. Regulation of proliferation in the 'DNA damage' S/G2 cell cycle checkpoint pathway is known for its role in the maintenance of chromatin structural integrity[4]. Here we show that autocrine/paracrine γ-aminobutyric acid (GABA) signalling by means of $GABA_A$ receptors negatively controls ES cell and peripheral neural crest stem (NCS) cell proliferation, preimplantation embryonic growth and proliferation in the boundary-cap stem cell niche, resulting in an attenuation of neuronal progenies from this stem cell niche. Activation of $GABA_A$ receptors leads to hyperpolarization, increased cell volume and accumulation of stem cells in S phase, thereby causing a rapid decrease in cell proliferation. $GABA_A$ receptors signal through S-phase checkpoint kinases of the phosphatidylinositol-3-OH kinase-related kinase family and the histone variant H2AX. This signalling pathway critically regulates proliferation independently of differentiation, apoptosis and overt damage to DNA. These results indicate the presence of a fundamentally different mechanism of proliferation control in these stem cells, in comparison with most somatic cells, involving proteins in the DNA damage checkpoint pathway.

Expression of subunits consistent with functional $GABA_A$ receptors ($GABA_A$Rs) was found in both ES cells positive for stage-specific embryonic antigen 1 (SSEA-1) and NCS cells derived from the boundary cap (BC)[5] (Fig. 1a and Supplementary Fig. 1) with a common and possibly critical expression of the β3 subunit. GABA or the $GABA_A$R-specific agonist muscimol evoked an outward current in whole-cell voltage-clamp recordings of ES cells held at −60 mV ($n = 15$) and the amplitude was decreased significantly by the $GABA_A$R-specific antagonist bicuculline (Fig. 1b and Supplementary Fig. 2a). With the use of the perforated-patch technique the resting membrane potential of ES cells was estimated as -26.05 ± 1.93 mV ($n = 20$; mean ± s.e.m.). The GABA reversal potential calculated by measuring the amplitude of the induced current at different holding potentials showed a linear relationship that reversed at −78 mV ($n = 11$) (Fig. 1c and Supplementary Fig. 2b). The patch clamp data were independently confirmed by using a voltage-sensitive dye, bis-(1,3-dibutylbarbituric acid)trimethine oxonol ($DiBAC_4(3)$), in ES and NCS cells (Supplementary Figs 2c, d and 3a). Ca^{2+} transients were not detected in NCS cells in response to $GABA_A$R activation (Supplementary Fig. 3b). Taken together, these data show that ES and NCS cells possess functional $GABA_A$Rs that induce a hyperpolarizing current by means of an influx of Cl^-.

Next we examined the significance of $GABA_A$R activation in stem cell proliferation. The rate of mitotic events was determined by exploiting the tight adhesion that occurs between daughter ES cells after mitosis, resulting in cell doublets that are identified by flow cytometry forward scattering (FSC) 2 h after dissociation (Fig. 1f). Muscimol decreased cell number (Fig. 1d,e) and mitotic events (Fig. 1f, g) in ES cell cultures and potently blocked the proliferation of NCS cells in a reversible manner (Fig. 1h and Supplementary Fig. 3c). The percentage of multipotent SSEA-1^+ ES cells and multipotency of NCS cells was unaffected, as were colony morphology and activity and the levels of markers for survival, death or differentiation in response to the agonist (Supplementary Fig. 4). The effect of muscimol seemed to be inversely proportional to cell numbers, because high-density cultures of both cell types showed a lower response. The density dependence of responses to the agonist suggested endogenous signalling. Expression analyses of components necessary for the production and release of GABA in ES and NCS cells showed their presence in both cell types (Fig. 1a and Supplementary Figs 1, 5 and 6); vesicular GABA transporter was localized in subcellular puncta (Fig. 2a and Supplementary Fig. 5). Differentiating ES cells (namely SSEA-1^- cells) rapidly lost expression of these markers, indicating that endogenous GABA signalling may be specific to stem cell populations (Supplementary Fig. 6). Inhibition of endogenous signalling with bicuculline in high-density ES cell cultures significantly increased cell numbers (Fig. 2b) and led to a marked increase in incorporation of bromodeoxyuridine (BrdU) into NCS cells (Fig. 2c, d). Suppression of $GABA_A$R β3 expression by RNA interference (RNAi; Supplementary Fig. 1i) led to an increase in ES cells without affecting colony morphology (Fig. 2e, f) and increased mitotic events in the 2 h cell doublet assay (Fig. 2g). These data indicate that endogenously synthesized GABA functions in an autocrine/paracrine manner in stem cells.

Consistent with unaffected cell cycle regulation at the G1–S transition (Supplementary Fig. 7a), the rapid effects of muscimol (Fig. 1f, g) suggested a mechanism acting within 2 h of mitosis; that is, in late S, G2 or M phase. BrdU incorporation during 1.5 h of muscimol stimulation showed a rapid threefold decrease in DNA synthesis (Fig. 2h). Cell cycle distribution analysis by flow cytometry revealed an accumulation or decrease in ES cells in S phase after 6 h of activation or inhibition of the receptor, respectively (Fig. 2i, j); this

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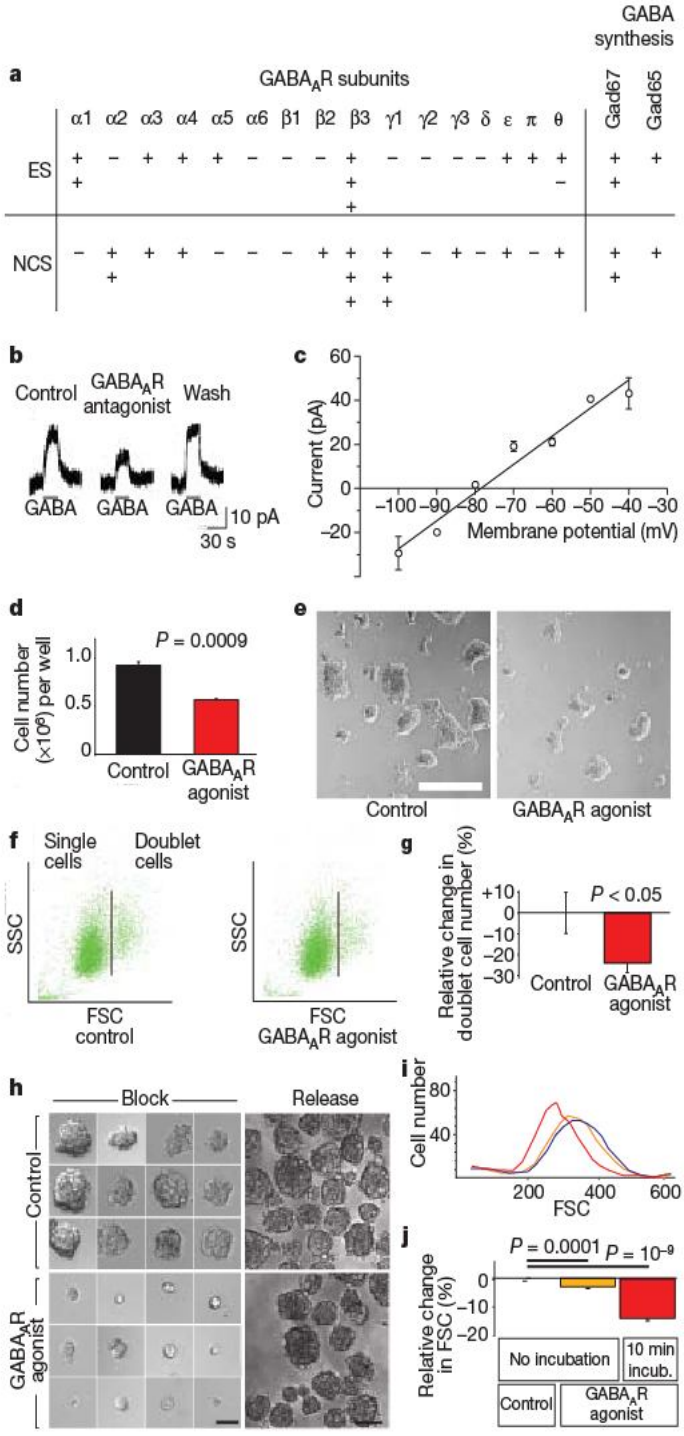

Figure 1 | Activation of expressed GABA$_A$R subunits results in hyperpolarization and has marked consequences on cell numbers in mouse ES and NCS cells. **a**, Expression analysis of GABA$_A$R subunits and synthesizing enzymes by real-time RT–PCR of SSEA-1$^+$ ES cells and NCS cells isolated by flow cytometry (+, ++, +++, expression levels; −, no expression). **b**, Electrophysiological analysis of R1 ES cells revealed an outward GABA-induced current that was reversibly blocked by the GABA$_A$ receptor antagonist bicuculline (100 μM). **c**, Reversal potential of the GABA-induced current (−78 mV) estimated by using the perforated-patch voltage-clamp technique and by stimulating with GABA at different holding potentials. **d**, ES cell numbers after 24 h of exposure to muscimol ($n = 3$). **e**, Photomicrograph of ES cell colonies in **d**. **f**, Flow cytometric analysis of forward and side scattering (FSC and SSC, respectively) of ES cells revealing the relative numbers of cell doublets reflecting mitotic events after 2 h of agonist treatment. **g**, Quantification of **f** ($n = 3$). **h**, Block-release experiment showing NCS cell spheres after a three-day treatment with or without muscimol and three days after release. **i**, **j**, Cell size measurement by FCS analyses with flow cytometry after muscimol. **i**, Distribution of cells within FSC levels. The colour code in **i** is the same as in **j**. **j**, Average of FSC levels revealing a rapid increase in cell size by GABA$_A$R activation ($n = 3$). Incub., incubation. Error bars indicate s.e.m. (two-tailed Student's *t*-test). Scale bars, 200 μm (**d**) and 25 μm (**g**).

was not observed in differentiated ES cells (Supplementary Fig. 6u). ES cells responded immediately to muscimol by decreasing FSC (Fig. 1i, j) which correlates with osmotic swelling[6]. Thus, an influx of Cl^- ions leads to a rapid increase in cell size followed by a control of proliferation by regulating cell cycle progression in S phase. ES cells within colonies responded synchronously to muscimol and showed dye coupling (Fig. 3c; $n = 6$). This suggests an electrochemical coupling that may allow hyperpolarizing currents to spread to neighbouring ES cells via gap junctions, coordinating the S-phase checkpoint pathway within a colony.

We analysed active phosphorylated histone H2AX (γ-H2AX), which is a critical factor of the S/G2 DNA-damage checkpoint complex[7], after exposing ES and NCS cells to muscimol. Muscimol rapidly increased γ-H2AX levels in nuclear foci of ES cells (Fig. 3a, b, d) which could be inhibited by antagonist (Fig. 3d). A significant increase in γ-H2AX was seen also in NCS cells (Supplementary

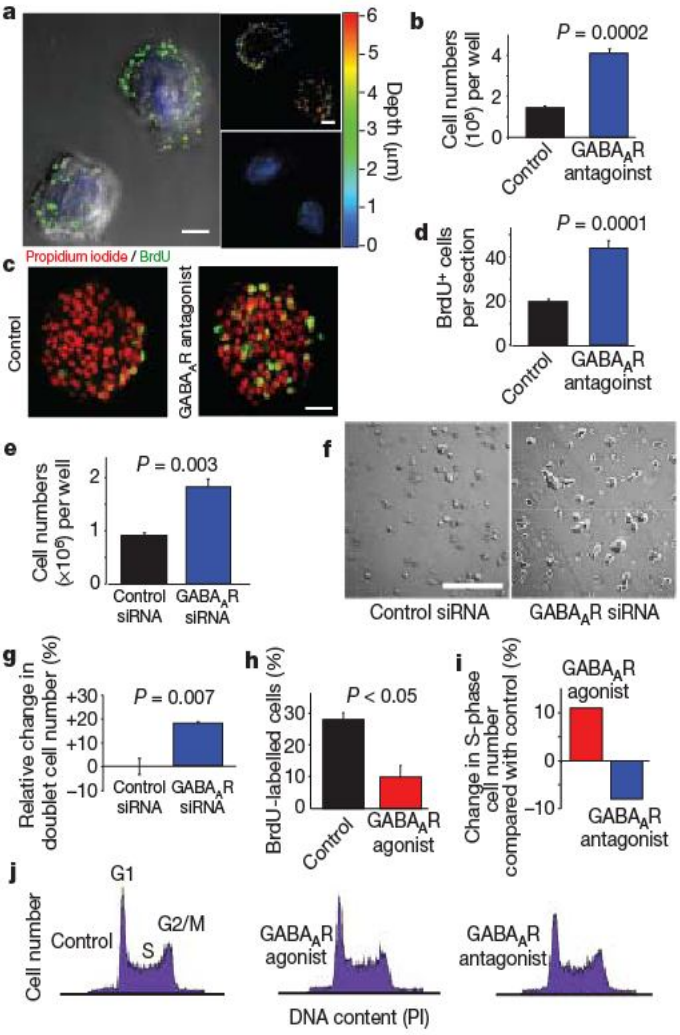

Figure 2 | Endogenous GABA$_A$ receptor signalling controls proliferation in ES and NCS cells. **a**, Immunofluorescence staining for vesicular GABA transporter (VGAT) in ES cells revealing a punctate localization (colour scale shows depth). **b**, Analysis of autocrine GABA signalling through the GABA$_A$R. ES cell numbers with bicuculline for two passages ($n = 3$) are shown. **c**, BrdU pulse (2 h) of NCS cells treated with bicuculline for 12 h. **d**, Quantification of **c** ($n = 74$ clones). **e**, ES cell numbers four days after transfection with siRNA against the GABA$_A$R β3 subunit ($n = 3$). **f**, Photomicrograph of ES cell colonies in **e**. **g**, ES cell proliferation 48 h after siRNA transfection by the 2-h doublet assay ($n = 3$). **h**, BrdU incorporation over the course of 1.5 h in ES cells exposed to muscimol ($n = 3$). **i**, Quantitative analysis showing the percent change of cells in S-phase based on flow cytometric cell cycle profile analyses following muscimol or antagonist (picrotoxin) for 6 h by flow cytometry. **j**, Cell cycle profiles of **i**. PI, propidium iodide. Error bars indicate s.e.m. (two-tailed Student's *t*-test). Scale bars, 5 μm (**a**), 75 μm (**c**) and 400 μm (**f**).

Fig. 3d). DNA tail comet assays revealed no overt DNA damage after 2 h of muscimol treatment (Fig. 3e, f). H2AX is phosphorylated by the phosphatidylinositol-3-OH kinase-related kinase (PIKK) family of kinases, including ataxia telangiectasia mutated (ATM), ataxia telangiectasia, Rad3-related (ATR) and DNA protein kinase (DNA-PK)[4]. In ES cells, short interfering RNA (siRNA) against ATR abolished the cell cycle distribution response to muscimol (Fig. 3g). ATR activates ATM in response to replication fork stalling[8]; we therefore analysed activated phosphorylated ATM (P-ATM). P-ATM was increased and decreased by muscimol and β3 siRNA, respectively (Fig. 3h, i). Furthermore, an ATM/DNA-PK inhibitor that blocked the phosphorylation and activity of ATM (Supplementary Fig. 7c, d) decreased γ-H2AX levels (Fig. 3d) and brought about a marked decrease in cells in S phase (Fig. 3j). The inability of the inhibitor to block all γ-H2AX (Fig. 3d) suggests a residual ATM/DNA-PK-independent component of H2AX activation, presumably by ATR. These results show that the PIKK family of kinases mediates $GABA_AR$ activation of H2AX in the absence of overt DNA damage.

The effect of GABA on cell proliferation was critically dependent on H2AX, because transfection of ES and NCS cells with siRNA against H2AX resulted in an increase in cell numbers that was not additive when both H2AX and $GABA_AR$ expression were decreased (Fig. 3k), and H2AX RNAi eliminated the effect of muscimol in NCS cells (Supplementary Fig. 3e) and ES cells in the 2-h cell doublet assay (Fig. 3l). Muscimol-induced γ-H2AX was consistently decreased in ES cells treated with β3 siRNA (Supplementary Fig. 7f). ES cell cycle distribution analysis revealed a marked decrease in cells in S phase with siRNAs (Fig. 3m and Supplementary Fig. 7b), similar to that with the ATM/DNA-PK inhibitor. This could be caused either by an increase in the time spent in other cell cycle phases or by an increased rate of progression of cells through S phase. β3, H2AX RNAi and ATM/DNA-PK inhibitor all markedly increased BrdU incorporation and decreased the proportion of cells at any given point in S phase (Fig. 3n, o), reflecting an increased rate of progression through S phase.

Next we addressed whether $GABA_AR$ activation controlled proliferation in the preimplantation embryo from which the ES cells were derived. Blastocysts responded rapidly to muscimol *ex vivo* by decreasing $DiBAC_4(3)$ fluorescence, which is consistent with hyperpolarization (Fig. 4a). *In vivo*, muscimol caused a marked decrease in BrdU incorporation in blastocysts (Fig. 4b). To investigate the physiological role of this during early development, $GABA_AR$ β3 subunit siRNA was injected into the cytoplasm of mouse zygotes, which developed into blastocysts *in vivo*. Blastocyst-stage embryos

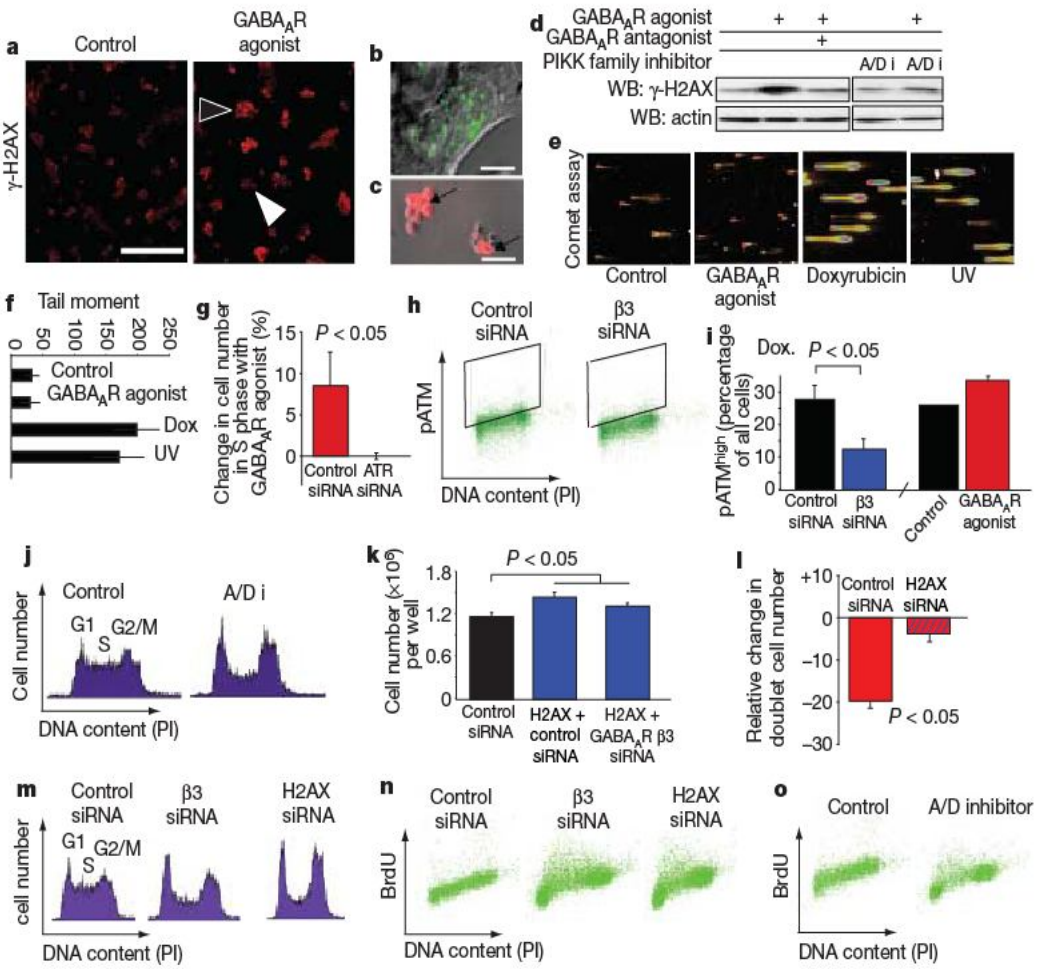

Figure 3 | $GABA_AR$ activation regulates S-phase cell cycle progression by activation of PIKK and phosphorylation of histone H2AX.
a, Immunostaining of ES cells for γ-H2AX after 6 h of exposure to muscimol. Open arrow, γ-H2AX$^+$ colony; filled arrow, baseline reactivity. **b**, Punctate nuclear localization of γ-H2AX staining in a single ES cell. **c**, Single ES cells filled with a gap-junction-permeable dye to reveal electrochemical coupling between cells within colonies; arrows indicate injected cells ($n = 6$). **d**, Western blot (WB) analysis of γ-H2AX in ES cells after exposure to muscimol or bicuculline and ATM/DNA-PK inhibitor (A/D i) for 6 h. **e**, Comet assay for DNA damage by 2 h of exposure to muscimol in ES cells. **f**, Quantification of **e**. **g**, Quantification of ATR RNAi-treated ES cells in S phase after 2 h of exposure to muscimol. **h**, **i**, Number of P-ATM^{high} cells by flow cytometric analysis after 2 h of exposure to muscimol in β3 subunit siRNA-treated cells. Dox., doxyrubicin. **j**, Cell cycle profiles of R1 ES cells with and without ATM/DNA-PK inhibitor (A/D i). **k**, ES cell numbers at 48 h with control siRNA or H2AX and control or H2AX and $GABA_AR$ β3 subunit siRNA ($n = 3$). **l**, Effects of muscimol on H2AX siRNA-treated ES cells in a 2-h cell doublet analysis ($n = 3$). **m**, Cell cycle profiles of R1 ES cells transfected with $GABA_AR$ β3 and H2AX siRNAs. **n**, Flow cytometric analysis of ES cells after a 50-min BrdU pulse. BrdU increases with β3/H2AX siRNA. **o**, Flow cytometric analysis of R1 ES cells treated with the ATM/DNA-PK (A/D) inhibitor. BrdU increases in the presence of inhibitor. Error bars indicate s.e.m. (two-tailed Student's *t*-test). Scale bars, 200 μm (**a**), 5 μm (**b**), 50 μm (**c**) and 100 μm (**e**).

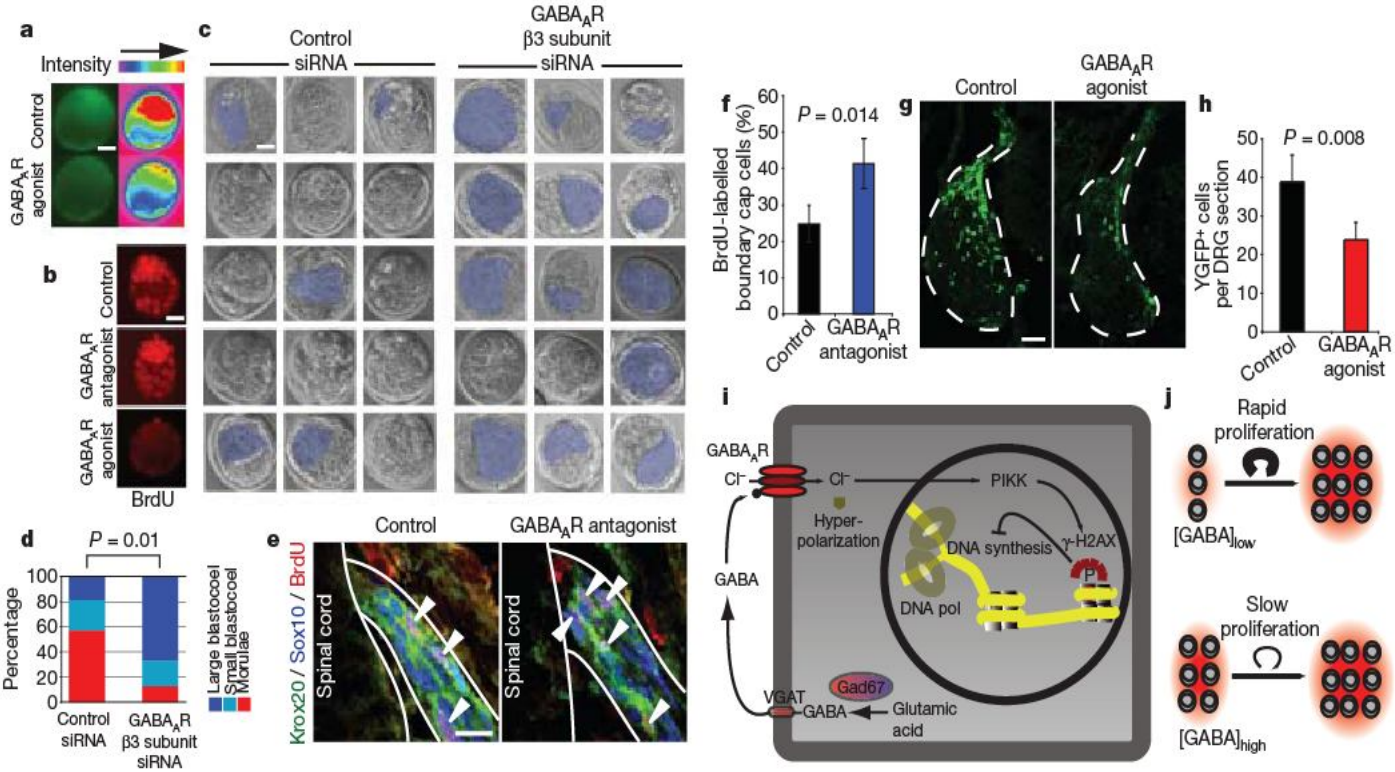

Figure 4 | *In vivo* role of GABA$_A$R signalling for embryo growth and BC stem cell niche progeny export. **a**, Membrane potentiometric analysis of blastocysts with $DiBAC_4(3)$ (1 μM) immediately before and 2 min after administration of GABA$_A$R agonist (muscimol). **b**, BrdU staining of blastocysts after 5 h of exposure *in vivo* to muscimol and a 1-h BrdU pulse. **c**, Zygote embryos microinjected with siRNA against GABA$_A$R β3 subunit were implanted into pseudopregnant recipients and developed into morula/blastocysts. Blue denotes blastocoel. **d**, Quantification of morulae and blastocysts with large or small blastocoels ($P < 0.01$, χ^2 test). **e**, BrdU and Sox10 staining of *Krox20*$^{eGFP/+}$ mice injected with bicuculline at E11 followed by a 2-h BrdU pulse 8 h later. There are fewer BrdU$^+$ (arrowheads) nuclei in the control than in the bicuculline-treated sample. **f**, Quantification of **e** (one-way analysis of variance, $n = 3$). **g**, Sections of DRG from *Krox20*$^{Cre/+}$ R26R-EYFP mice killed at E14 that received muscimol between E11 and E14. **h**, Quantification of **g**. Lineage tracing shows a significant decrease in the number of progeny from the BC in the DRG of treated mice (one-way analysis of variance, $n = 4$). Error bars indicate s.e.m. (two-tailed Student's *t*-test). **i**, Proposed model for GABA-mediated control of proliferation by means of chromatin modification in stem cells. ES and NCS cells synthesize physiological levels of GABA, which activate GABA$_A$Rs and cause hyperpolarization through Cl^- influx. GABA signals by means of PIKKs and activates histone H2AX by phosphorylation of Ser 139 to decrease DNA synthesis and cell cycle progression. **j**, Proposed consequences of GABA signalling in stem cells. GABA levels correlate with stem cell numbers, which results in an inverse correlation between proliferation rate and stem cell pool size. Scale bars, 25 μm (**a**, **b**), 20 μm (**c**), 10 μm (**e**) and 50 μm (**g**).

exposed to β3 siRNA developed faster than controls and contained smaller cells and a larger blastocoel (Fig. 4c, d). These results show that proliferation and growth of the preimplantation embryo is regulated by GABA$_A$R signalling, similarly to ES cells.

NCS cells are derived from the BC stem cell niche located at the border between the peripheral nervous system and the central nervous system[5,9] and produce peripheral sensory neurons and glia[10]. To test whether GABA$_A$R activation regulates proliferation in this population *in vivo*, bicuculline was administered to *Krox20*$^{eGFP/+}$ mice expressing the fluorophore enhanced green fluorescent protein (EGFP) specifically in BC cells at embryonic day 11 (E11) (ref. 10). Despite a high rate of proliferation, with more than 20% of the cells incorporating BrdU after a 2-h pulse, blocking endogenous GABA signalling led to a nearly twofold increase in incorporation (Fig. 4e, f). Mice expressing a knock-in allele for the Cre recombinase in the *Krox20* locus (*Krox20*$^{Cre/+}$)[11,12] crossed with a ROSA26 reporter (R26R) mouse strain permanently activating expression of the fluorophore enhanced yellow fluorescent protein (EYFP) on recombination[10,13] was used to trace the cell lineage of BC progenies genetically. Consistent with previous findings was the observation of numerous EYFP$^+$ cells within the dorsal root entry zone, at motor exit points and in the dorsal root ganglion (DRG) in E14 sections at thoracic levels (Fig. 4g)[10]. The administration of muscimol to pregnant mice between E11 and E14 led to a marked decrease in EYFP$^+$ BC-derived cells (Fig. 4g, h). These results show that GABA signalling controls the number of cells produced from the BC stem cell niche during development.

Our results (Fig. 4i, j) show that endogenously produced GABA acting through PIKK-family proteins and H2AX has the following functions: first, it regulates ES and NCS cell proliferation; second, it determines early embryo size; and third, it determines proliferation in the BC stem cell niche *in vivo*, resulting in direct consequences on progeny cell number. Unlike conventional G1-phase cell cycle control, which is linked to cell differentiation, this S-phase mechanism may provide a way to control proliferation independently of differentiation in the stem cells. Induction of the PIKK/H2AX pathway seems to be independent of DNA damage, in a similar manner to that previously described during sex chromosome inactivation[14], hypoxia[15] and mitosis[16,17]. We find it intriguing that ES and NCS cells share a similar mechanism regulating their proliferation, especially in view of the reported effects of GABA on the proliferation of adult brain progenitor cells that are positive for glial fibrillary acidic protein[18]. Thus, seemingly disparate biological processes may be affected by a common mechanism of control of stem cell proliferation.

METHODS SUMMARY

The EB5 and R1 ES strains were cultured in knockout serum replacement and leukaemia inhibitory factor (LIF) or as described[19], and NCS cells were cultured as described[5]. Antibody against SSEA-1 or γ-H2AX together with secondary antibody was used for flow cytometry analysis on a FACScan instrument and cell sorting on a FACSVantage SE/FACS DiVa instrument (Becton Dickinson). Quantification was performed with CellQuest Pro software (Becton Dickinson). Immunostainings, real-time RT–PCR and biochemical analyses were performed in accordance with standard procedures. Two siRNAs (27-mers) were designed for each mRNA and transfected at 5 μM. Control siRNA was used at equimolar

levels. For *in vivo* analyses, zygotes were injected into the cytoplasm with 10 pl of 20 μM siRNA. Picrotoxin, bicuculline and muscimol were used at 50, 20 and 100 μM, respectively, on cultured cells with controls receiving vehicle. For experiments *in vivo*, bicuculline or muscimol was diluted in PBS and administered intraperitoneally to pregnant females at 4 mg kg^{-1}; for long-term experiments this dose was given twice a day. BrdU (50 mg kg^{-1}, in PBS) was administered intraperitoneally. Mice were held on a pure C57/Bl6 or mixed C57Bl6/DBA2 background[10]. ATM/DNA-PK inhibitor (Calbiochem) was used at 10 μM.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions M.A. was the main contributor to the work on ES cells; J.H.-L. was the main contributor to the work on NCS cells.

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LETTERS

Dscam and Sidekick proteins direct lamina-specific synaptic connections in vertebrate retina

Masahito Yamagata[1] & Joshua R. Sanes[1]

Synaptic circuits in the retina transform visual input gathered by photoreceptors into messages that retinal ganglion cells (RGCs) send to the brain. Processes of retinal interneurons (amacrine and bipolar cells) form synapses on dendrites of RGCs in the inner plexiform layer (IPL). The IPL is divided into at least 10 parallel sublaminae; subsets of interneurons and RGCs arborize and form synapses in just one or a few of them[1–3]. These lamina-specific circuits determine the visual features to which RGC subtypes respond[3–5]. Here we show that four closely related immunoglobulin superfamily (IgSF) adhesion molecules—Dscam (Down's syndrome cell adhesion molecule), DscamL (refs 6–9), Sidekick-1 and Sidekick-2 (ref. 10)—are expressed in chick by non-overlapping subsets of interneurons and RGCs that form synapses in distinct IPL sublaminae. Moreover, each protein is concentrated within the appropriate sublaminae and each mediates homophilic adhesion. Loss- and gain-of-function studies *in vivo* indicate that these IgSF members participate in determining the IPL sublaminae in which synaptic partners arborize and connect. Thus, vertebrate Dscams, like *Drosophila* Dscams[11–19], play roles in neural connectivity. Together, our results on Dscams and Sidekicks suggest the existence of an IgSF code for laminar specificity in retina and, by implication, in other parts of the central nervous system.

We previously showed that Sidekick-1 and Sidekick-2, large transmembrane adhesion molecules of the IgSF, are expressed by complementary subsets of amacrine, bipolar and ganglion cells in chick retina[10]. Double-label *in situ* hybridization revealed that only 10–15% of RGCs were positive for each *Sidekick* (Supplementary Table 1). We speculated that Sidekick-negative retinal neurons might express genes encoding related molecules. Homology searches of genomic databases revealed that their closest relatives are the Dscams[6–9], which, like Sidekicks, contain multiple immunoglobulin and fibronectin type III domains, plus a carboxy terminus predicted to bind PDZ domains (Fig. 1a and Supplementary Fig. 1). Moreover, vertebrate Dscams are related to *Drosophila* Dscams, which have been implicated in neural specificity[11–19], although they lack the complex pattern of alternative splicing found in *Drosophila Dscam-1*. We therefore cloned chick *Dscam* and *DscamL*, the orthologues of mammalian *Dscam* and *Dscam-like-1*, respectively, and assessed their expression in retina. Dscam and DscamL, like Sidekicks, were expressed by non-overlapping subsets of retinal neurons between embryonic day (E) 12 and hatching (E21) (Fig. 1b and Supplementary Fig. 2). None of the *Dscam*-positive neurons expressed either *Sidekick* (Fig. 1c and Supplementary Fig. 3). Thus, *Dscams* and *Sidekicks* mark four distinct sets of presynaptic (amacrine and bipolar) and postsynaptic cells (RGCs) that arborize in the IPL.

To localize Dscam and Sidekick proteins, we generated antibodies specific for each (Supplementary Fig. 4) and used them to immunostain retinal sections. We follow the convention[1–3] of dividing the IPL into 5 parallel slabs of equal width, S1–S5; each, however, may contain 2 or more sublaminae defined physiologically[4,5]. Each IgSF protein was concentrated in a distinct set of IPL sublaminae: Dscam in S5, DscamL in S1, S2 and S4, Sidekick-1 predominantly in the inner half of S4 with low expression in the inner half of S2, and Sidekick-2 predominantly in the inner half of S2 with low expression in the inner half of S4 (Fig. 1d). Confocal imaging showed that Dscams were frequently associated with vesicle-rich synaptic sites in the IPL (not shown), as shown previously for Sidekicks[10]. *In situ* hybridization of retinae in which individual cells had been filled with GFP (green fluorescent protein) by transposon-mediated gene transfer (see below) showed that neurites of cells expressing each Dscam or Sidekick gene arborize in IPL sublaminae containing the corresponding Dscam or Sidekick protein (Supplementary Fig. 5, ref. 10 and data not shown; summarized in Fig. 1e). Thus, Dscams and Sidekicks mark four distinct synaptic pathways in the retina.

Dscams, like Sidekicks, might not only mark lamina-specified neuronal subsets but also play roles in establishing or maintaining the sublaminar specificity of their synapses. We predicted that if this were the case, (1) each Dscam and Sidekick would mediate homophilic but not heterophilic adhesion; (2) depletion of Dscam or Sidekick would selectively perturb arbors of neurons in particular IPL sublaminae; and (3) ectopic expression of a Dscam or Sidekick would reroute arbors of the expressing cell to normally Dscam- or Sidekick-positive IPL sublaminae. We tested these ideas in turn.

To assess adhesion, we transfected heterologous cells with complementary DNAs encoding Dscams or Sidekicks, then allowed them to aggregate. Cells expressing any one of these four IgSF members formed aggregates but untransfected cells did not (Fig. 2a and data not shown). Moreover, Dscam and Sidekick proteins became concentrated at sites of contact between transfected cells (Fig. 2b and data not shown). Thus, as shown previously[7,8,10], Dscams and Sidekicks mediate homophilic adhesion. To assess heterophilic adhesion, we labelled transfected populations separately with red and green fluorescent dyes. When both populations expressed the same adhesion molecule, red and green cells co-aggregated to form mixed clusters, but when the two populations expressed different adhesion molecules, mixed aggregates were rare (Fig. 2a, c). Thus Dscams and Sidekicks do not detectably interact with each other under the conditions of our assay.

To decrease Dscam and Sidekick levels in retinal neurons, we identified interfering RNAs[20,21] that targeted Dscam, Sidekick-1 and Sidekick-2 effectively (Supplementary Fig. 6) and incorporated them into retroviral vectors[22] that also expressed GFP (Fig. 3a and b). Infection of retina with these vectors selectively decreased expression of the corresponding protein (Supplementary Fig. 7a). To assess effects of depleting Dscam or Sidekicks, we needed independent markers of the neurons that normally express them, so we could

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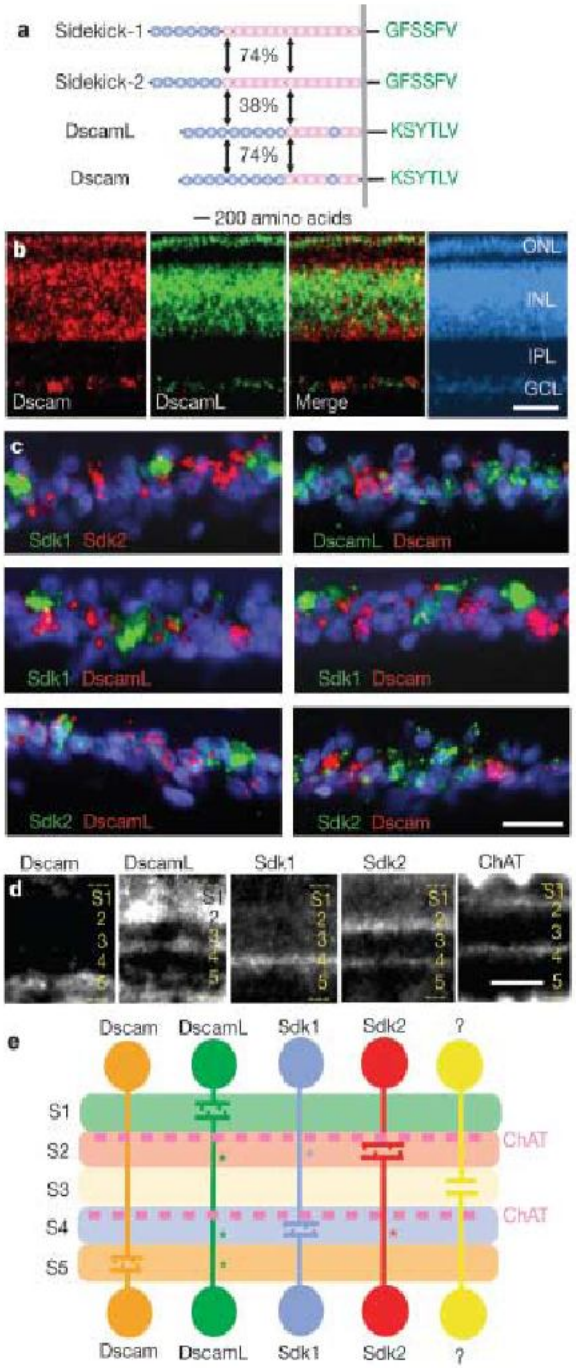

Figure 1 | Expression and distribution of Dscams and Sidekicks in retina. **a**, Structure of Dscam, DscamL and Sidekick (Sdk)-1 and -2. Blue, immunoglobulin domains; pink, fibronectin type III domains; grey, membrane; green, carboxy-terminal sequences predicted[6–10] and shown (M.Y., unpublished data) to bind PDZ domains. Numbers show percentage amino acid similarity between indicated pairs of proteins, based on the Smith-Waterman algorithm applied to the entire sequence (see Methods). **b**, **c**, Double label *in situ* hybridization to sections of E15 retina showing that Dscam, DscamL, Sidekick-1 and Sidekick-2 are expressed by non-overlapping subsets of neurons. Whole retina is shown in **b**, ganglion cell layer only in **c**. Nuclear stain, DAPI, shown in blue. (See also Supplementary Fig. 3.) **d**, Immunostaining of retina with antibodies specific for Dscams, Sidekicks and choline acetyltransferase (ChAT). Each is concentrated in distinct sublaminae of the IPL. **e**, Summary of results from **b–d** and Supplementary Figs 2, 3 and 5, showing that each Dscam and Sidekick is concentrated in IPL sublaminae in which cells expressing the cognate gene form synapses. Predominant IPL sublamina marked by each IgSF member is drawn; sites of lower expression are shown with asterisks. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; S1–S5, subdivisions of the IPL. '?' indicates that many retinal neurons are both Sidekick- and Dscam-negative, and may express other IgSF genes. Scale bars: **b**, 50 μm; **c**, 25 μm; and **d**, 20 μm.

identify appropriate cells following depletion. We tested previously described markers of RGC subsets[10,23,24], and found that most Dscam-, Sidekick-1- and Sidekick-2-positive RGCs also expressed R-cadherin, cadherin-7 and calbindin, respectively (Supplementary Fig. 8 and data not shown). Thus, we could use these markers to assess lamina-specific connectivity in the absence of Dscam or Sidekicks.

Depletion of Dscam disrupted the laminar patterning of R-cadherin-positive processes in S5. Likewise, depletion of Sidekick-1 and Sidekick-2 disrupted laminar patterning of cadherin-7- and calbindin-positive processes, respectively, in S4. In all three cases, processes appeared defasciculated and extended beyond the boundaries of the sublamina in which they were normally confined (Fig. 3c). Several observations indicated that these effects were specific (Fig. 3c, Supplementary Fig. 7 and data not shown). First, no disruptions were seen in uninfected areas of the same retinae. Second, depletion of each IgSF member affected only the RGC subset marked by the coexpressed gene. Third, we saw no displacement of processes formed by choline acetyltransferase- and substance P-positive cells, which do not express Dscam or either Sidekick, although a small number of choline acetyltransferase-positive somata were sometimes displaced into the IPL following Dscam depletion (Supplementary Fig. 9). Fourth, R-cadherin and cadherin-7 were present not only in Dscam- and Sidekick-1-positive processes in S4 and S5, but also in Dscam- and Sidekick-1-negative processes in S2; only the former were affected by the interfering RNAs (see Supplementary Fig. 7 for examples and quantification). Finally, similar results were obtained with each of two interfering RNAs that depleted Dscam (Supplementary Fig. 6). Thus, Dscam, Sidekick-1 and Sidekick-2 are necessary for lamina-specific arborization of neurites in the IPL (Fig. 3d).

Next, we asked whether expression of Dscams or Sidekicks was sufficient to specify the laminar position of neurites in the IPL. Retroviral vectors such as those used to deliver interfering RNAs

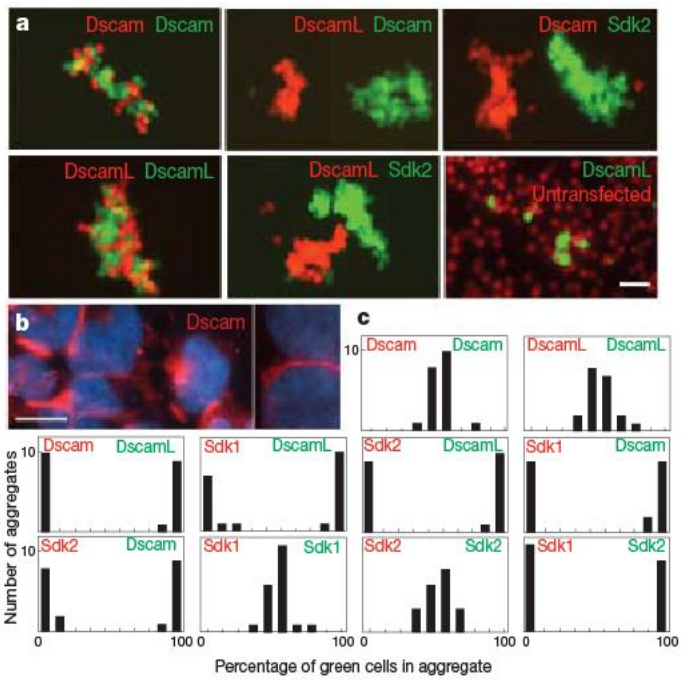

Figure 2 | Dscams and Sidekicks mediate homophilic adhesion but do not adhere to each other. Cells expressing each IgSF member were labelled red or green and incubated for 1 h. The extent to which cells of the two colours mixed within aggregates was measured. **a**, Examples of homophilic but not heterophilic adhesion. Untransfected cells did not aggregate under these conditions. **b**, Dscam concentrates at sites of cell–cell apposition; low power figure at left, high power (twice magnified) at right. Nuclei marked with DAPI (blue). **c**, Percentage of green cells in each of 20 aggregates from each of 10 red-green combinations. Scale bars: **a**, 50 μm; **b**, 10 μm (left) and 5 μm (right).

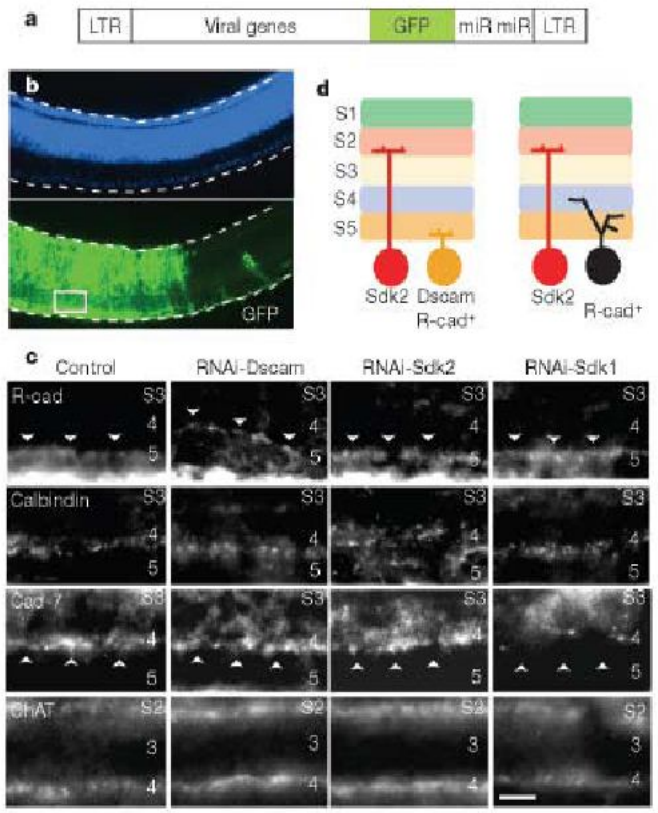

Figure 3 | Depletion of Dscam or Sidekick perturbs lamina-specific arborization. **a**, Retroviral vector. LTR, long terminal repeat; GFP, green fluorescent protein; miR, artificial micro-RNAs directed at Dscam or Sidekick. **b**, Section from an E17 retina following retroviral introduction at E2. Large patches of retinal cells are infected, as shown by GFP expression (green). Blue, DAPI. Rectangle indicates approximate size and location of areas shown in **c**. **c**, IPL in areas infected by virus carrying control, Dscam, Sidekick-1 or Sidekick-2 interfering RNA, then immunostained as indicated with markers selectively coexpressed by RGCs that express Dscam (R-cadherin), Sidekick-1 (cadherin-7) and Sidekick-2 (calbindin). Depletion of Dscam, Sidekick-1 and Sidekick-2 leads to selective mislocalization of R-cad-positive processes in S5, and cadherin-7- and calbindin-positive processes in S4, respectively. Quantification of effects is detailed in Supplementary Fig. 7 legend. **d**, Summary of RNAi results, illustrated for Dscam. Scale bar in **c** indicates 50 μm for **b** and 3 μm for **c**.

could not be used for this purpose because the size of the required cassette (8–9 kb) exceeds the packaging capacity of available vectors[22]. Electroporation of plasmids[10] was also problematic because expression levels declined greatly during the 2-week interval between gene transfer (on E2) and scoring (on E17). We therefore used an alternative method of gene transfer utilizing PiggyBac transposon and transposase[25,26]. We adapted this insect-derived system to chicks, generated transposons encoding Dscams or Sidekicks plus GFP (Fig. 4a), introduced them into retina, and scored the laminar distribution of GFP-positive processes emanating from groups of GFP-expressing cells. When only GFP was introduced, processes were evenly distributed among IPL sublaminae, but introduction of a

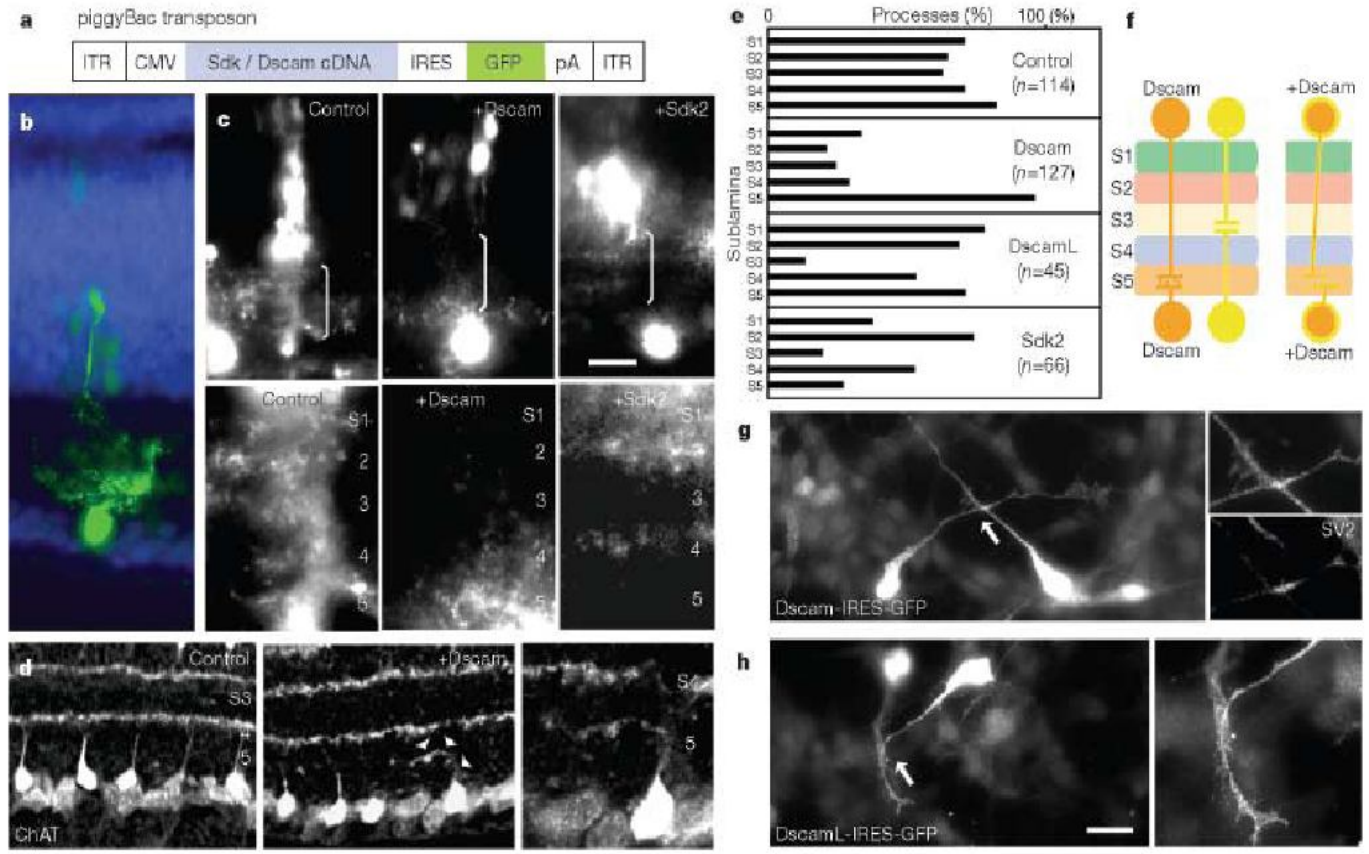

Figure 4 | Ectopic expression of Dscams or Sidekicks redirects lamina-specific arbors. **a**, PiggyBac transposon vector used to introduce Dscam or Sidekick plus GFP into retina. CMV, cytomegalovirus promoter and enhancer; ITR, internal terminal repeat; IRES, internal ribosomal re-entry site; pA, polyadenylation signal. **b**, GFP-labelled cells in E17 retina following electroporation at E2. Blue, DAPI. **c**, GFP-labelled processes were distributed in all sublaminae when GFP alone was overexpressed. Ectopic expression of Dscam or Sidekick-2 shifted processes to Dscam-positive S5 or Sidekick-2-positive S2, respectively. Brackets in top panels mark regions magnified in lower panels. **d**, Sections from retinae transfected with Dscam-GFP or GFP alone, stained for ChAT, which is expressed by a population of Dscam-negative amacrine cells. Dscam diverts ChAT-positive processes from S2 and S4 to S5. Right panel shows region marked by arrowheads in centre panel. **e**, Laminar position of GFP-positive processes in electroporated retinae. Areas like those shown in **c** (n = 45–127 per construct) were analysed. **f**, Summary of gain-of-function results, illustrated for Dscam. **g**, **h**, Cultured neuronal cells were transfected with plasmids encoding Dscam-IRES-GFP (**g**), or DscamL-IRES-GFP (**h**) then stained with antibodies to GFP and the synaptic vesicle protein SV2. Regions indicated by arrows are shown at higher magnification at right. Neurites from transfected cells contacted each other, and sometimes accumulated SV2 at such points of contact or fasciculated with each other. Scale bar in **c** indicates 25 μm for **b**, 50 μm for **c** (top panels), 10 μm for left and centre panels in **d**, and 3.3 μm for right panel in **d**. Bar in **h** indicates 20 μm for left panels in **g** and **h**, and 10 μm for right panels in **g** and **h**.

Dscam or Sidekick diverted processes to the sublamina in which the corresponding endogenous adhesion molecule is normally concentrated (Fig. 4b–e and data not shown). Cells ectopically expressing Dscam, for example, predominantly arborized in S5 (Fig. 4e). At least some of these processes had been destined for Dscam-negative laminae, as evidenced by their expression of choline acetyltransferase, which is normally confined to S2 and S4 (Fig. 4d). Likewise, cells that ectopically expressed DscamL avoided S3, which is normally DscamL-negative, and cells that ectopically expressed Sidekick-1 or Sidekick-2 sent processes predominantly to S2 and S4 (Fig. 4e, ref. 10 and data not shown). In all cases, both RGCs and interneurons were affected. Thus Dscams and Sidekicks are sufficient to strongly bias the laminar choices of retinal neurons (Fig. 4f).

These results suggest that Dscams and Sidekicks act as attractive or adhesive cues in the IPL, whereas *Drosophila* Dscams act as repulsive factors[14,17–19]. To explore this difference, we expressed Dscams or Sidekicks, along with GFP, in cultured neural cells. The transfected cells extended neurites that contacted each other, often accumulated synaptic vesicles at sites of contact, and sometimes fasciculated (Fig. 4g and h and Supplementary Fig. 10). The difference between the behaviour of vertebrate and *Drosophila* Dscams is likely to reflect context-dependence of signalling; many family of guidance molecules, including ephrins and netrins, act as attractants in some cells and repellents in others[27,28].

In summary, we have shown that Dscams and Sidekicks mark and help to specify four parallel pathways through the inner retina. They mediate homophilic adhesion and are both necessary and sufficient to ensure sublamina-specific arborization in the IPL. We do not yet know the steps at which they act; they might regulate arborization of pre- and postsynaptic processes, target recognition, or stabilization of synaptic contacts. Although positioning of some amacrine cells is perturbed in Dscam mutant mice[29], we do not believe that the defects we have seen are indirect consequences of such perturbations, in that RNA-interference-mediated loss of Dscam in chick does not lead to significant alterations in the distribution of amacrine cells that normally express this gene (data not shown). Moreover, positioning of amacrines is normal in early postnatal mouse Dscam mutants, at the time that synapses are forming in the IPL[29]. Although Dscam may play slightly different roles in the two species, we favour the idea that it has distinct effects on the two processes.

Dscams and Sidekicks mark only ~60% of RGCs and a similar fraction of retinal interneurons (Supplementary Fig. 3 and Supplementary Table 1). Other adhesion molecules may exist that mark Dscam- and Sidekick-negative retinal neurons, subdivide groups of cells expressing each of these four IgSF members, and specify the tectal sublaminae in which axons of defined RGC subsets[24] terminate. The retinotectal system may be useful for testing the idea that a complex network of recognition molecules mediates lamina-specific synaptic connectivity. Importantly, laminar specificity is a common feature of many circuits in the vertebrate brain and spinal cord[30]. As Dscams and Sidekicks are expressed by neuronal subsets in the brain (refs 6 and 9, and M.Y., unpublished results), similar networks may be used beyond the retina.

METHODS SUMMARY

Reagents. Monoclonal antibodies to chick Dscam and DscamL were generated using bacterially expressed recombinant proteins as immunogens. Full length cDNAs encoding Dscams and Sidekicks were cloned as described in Methods. For RNA interference, the DNA sequences specified in Methods were inserted along with GFP into the retroviral vector RCAS-BP (B). PiggyBac plasmids[25] were obtained from M. J. Fraser (University of Notre Dame) and modified as described in Methods.

Histology. *In situ* hybridization and immunostaining were performed as described previously[10,24].

Gene transfer *in ovo*. RCAS plasmids (for loss-of-function assays) or a 10:1:1 mixture of transposon, transposase and dsRed plasmids (for gain-of-function assays) were injected into the optic vesicles of stage 9–11 chick embryos. Electroporation was with three square pulses of 7 V, 25 ms. Embryos were fixed and sectioned at E17.

Cell culture. For adhesion assays, HEK293 cells (ATCC) were transfected with plasmids encoding Sidekick- or Dscam-GFP fusion proteins (EGFP-N1, Clontech). Stably expressing cells were selected, labelled with Cell tracker dyes (Invitrogen), and rotated at room temperature for 1 h. For assays of neurite outgrowth, human neuroblastoma cells (SH-SY 5Y; ATCC) were plated on glass cover slips, transfected with Sidekick- or Dscam-IRES-GFP plasmid, then fixed and immunostained 3 days later.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions M.Y. and J.R.S. conceived the experiments. M.Y. performed the experiments. J.R.S. and M.Y. wrote the paper.

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LETTERS

Neurite arborization and mosaic spacing in the mouse retina require DSCAM

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To establish functional circuitry, retinal neurons occupy spatial domains by arborizing their processes, which requires the self-avoidance of neurites from an individual cell, and by spacing their cell bodies, which requires positioning the soma and establishing a zone within which other cells of the same type are excluded[1]. The mosaic patterns of distinct cell types form independently and overlap. The cues that direct these processes in the vertebrate retina are not known[2,3]. Here we show that some types of retinal amacrine cells from mice with a spontaneous mutation in Down syndrome cell adhesion molecule (*Dscam*), a gene encoding an immunoglobulin-superfamily member adhesion molecule[4,5], have defects in the arborization of processes and in the spacing of cell bodies. In the mutant retina, cells that would normally express *Dscam* have hyperfasciculated processes, preventing them from creating an orderly arbor. Also, their cell bodies are randomly distributed or pulled into clumps rather than being regularly spaced mosaics. Our results indicate that mouse DSCAM mediates isoneuronal self-avoidance for arborization and heteroneuronal self-avoidance within specific cell types to prevent fasciculation and to preserve mosaic spacing. These functions are analogous to those of *Drosophila* DSCAM (ref. 6) and DSCAM2 (ref. 7). DSCAM may function similarly in other regions of the mammalian nervous system, and this role may extend to other members of the mammalian *Dscam* gene family.

We have identified a spontaneous mutation in mice that creates a loss-of-function allele of *Dscam*, the *Drosophila* homologues of which function in both isoneuronal self-avoidance for dendrite arborization and heteroneuronal self-avoidance for axon tiling[7–12]. The recessive mutation arose in the BALB/cByJ genetic background and caused an overt neurological phenotype. Mutant and wild-type mice are indistinguishable at birth, but are severely uncoordinated by postnatal day 3 (P3); as adults, the mutant mice have spontaneous seizures and kyphosis, but are fertile and long-lived (>24 months; see http://www.jax.org/research/media/wild_type.html for video). Through positional cloning (see Methods), a mutation was identified in *Dscam*. Sequencing of genomic and complementary DNA from affected mice revealed a 38-bp deletion in exon 17, causing a frame shift resulting in ten unique amino acids followed by a premature stop codon (Supplementary Fig. 1). This mutation truncates the protein in the second fibronectin repeat (Fig. 1a). *Dscam* messenger RNA levels in the brain were reduced by 70% in affected mice, consistent with nonsense-mediated decay (Fig. 1b).

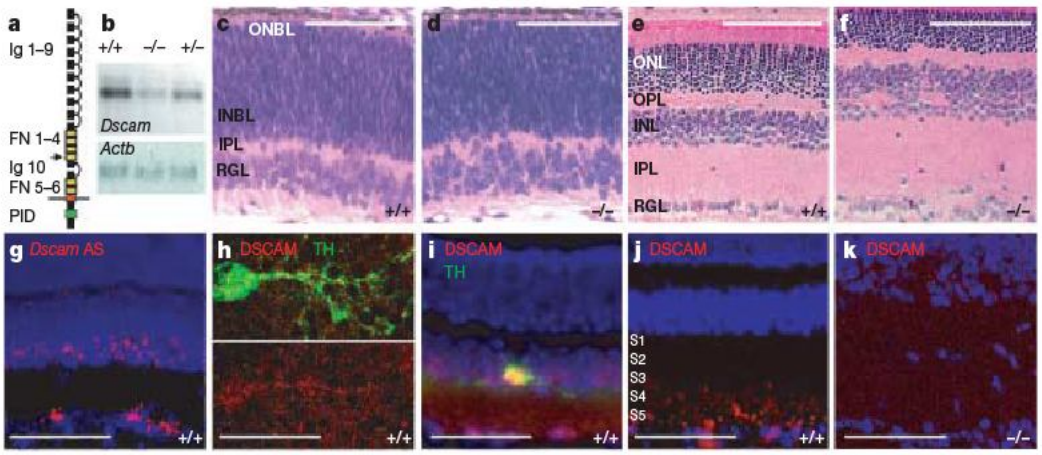

Figure 1 | Identification of a mouse *Dscam* mutation. **a**, A schematic of the DSCAM protein domain structure. The extracellular portion of DSCAM consists of ten immunoglobulin-like repeats (Ig) and six fibronectin domains (FN). The *Dscam* deletion truncates the coding sequence in the second fibronectin domain (arrow), which is before the transmembrane and PAK1-interacting domains (PID). **b**, Northern blotting of mRNA purified from whole brains of wild-type (+/+), $Dscam^{+/-}$ and $Dscam^{-/-}$ mice revealed a 70% reduction in *Dscam* mRNA in the mutant sample. β-actin (*Actb*) was used as a loading control. **c–f**, Haematoxylin and eosin stained sections of $Dscam^{-/-}$ and wild-type retinas from P0 and adult mice. The $Dscam^{-/-}$ retina is indistinguishable from that of the wild type during embryonic stages of development and at birth (**c**, **d**). INBL, innerneuroblast layer; ONBL, outerneuroblast layer; ONL, outernuclear layer; OPL, outerplexiform layer. **e**, **f**, In the adult $Dscam^{-/-}$ retina, the inner nuclear (INL), inner plexiform (IPL) and retinal ganglion (RGL) layers are disorganized compared to those of the wild-type retina, whereas other retinal layers are indistinguishable from that of the wild type. **g**, *In situ* hybridization with *Dscam* antisense probes (*Dscam* AS) revealed expression in a subset of cells in the inner nuclear and ganglion cell layers. **h**, Whole control P15 retina stained with antibodies to DSCAM and TH. **i**, A section of a control adult (6–10-week-old) retina labelled using antibodies to DSCAM and TH. **j**, A section of wild-type P15 retina stained with DSCAM antibodies. **k**, A section of the $Dscam^{-/-}$ retina stained with an antibody to DSCAM. Scale bars: **c**, **d**, 80 μm; **e**, **f**, 106 μm; **g**, 160 μm; **h**, 45 μm; **i**, 120 μm; **j**, **k**, 65 μm.

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Despite the overt neurological phenotype of *Dscam*$^{-/-}$ mice, examination of the central nervous system of adults and developing embryos did not reveal any gross disorganization, with the exception of a caudal folium of the cerebellum (Supplementary Fig. 2), implying a subtle phenotype or a subpopulation of affected cells.

We did find disorganization of postnatal retinal anatomy (Supplementary Fig. 3). At birth or before, the mutant retina was indistinguishable from that of control mice (Fig. 1c, d). However, by P4, the ganglion cell layer and developing inner plexiform layer (IPL) were disorganized (not shown). This disorganization persisted into adulthood in amacrine and ganglion cells (Fig. 1e, f), and was confirmed with marker analysis (Supplementary Fig. 4). Within these cell populations, many subtypes are present[13,14], and *Dscam* was expressed in a subset of cells in these layers (Fig. 1g). Double *in situ* hybridization with markers of defined amacrine subtypes indicated that *Dscam* was expressed in dopaminergic amacrine cells (tyrosine hydroxylase (*Th*)-positive) and in *bNOS*-positive (*Nos1*-expressing) cells, but not in choline acetyltransferase (*Chat*)-positive starburst amacrine cells (SACs) or disabled 1-positive (*Dab1*) AII amacrine cells (Supplementary Fig. 5). Immunofluorescence with an antibody against the extracellular domain showed that DSCAM completely overlapped dopaminergic amacrine cell staining in >400 cells examined. In whole-mount retinas at P15, anti-DSCAM staining was distributed across the soma and processes of dopaminergic amacrine cells, but redistributed to the cell body in the adult retina (Fig. 1h, i). Additional immunoreactivity was detected in retinal cross-sections in lower, more vitreal layers of the IPL, below the strata of TH-positive processes (Fig. 1j). DSCAM immunoreactivity was absent

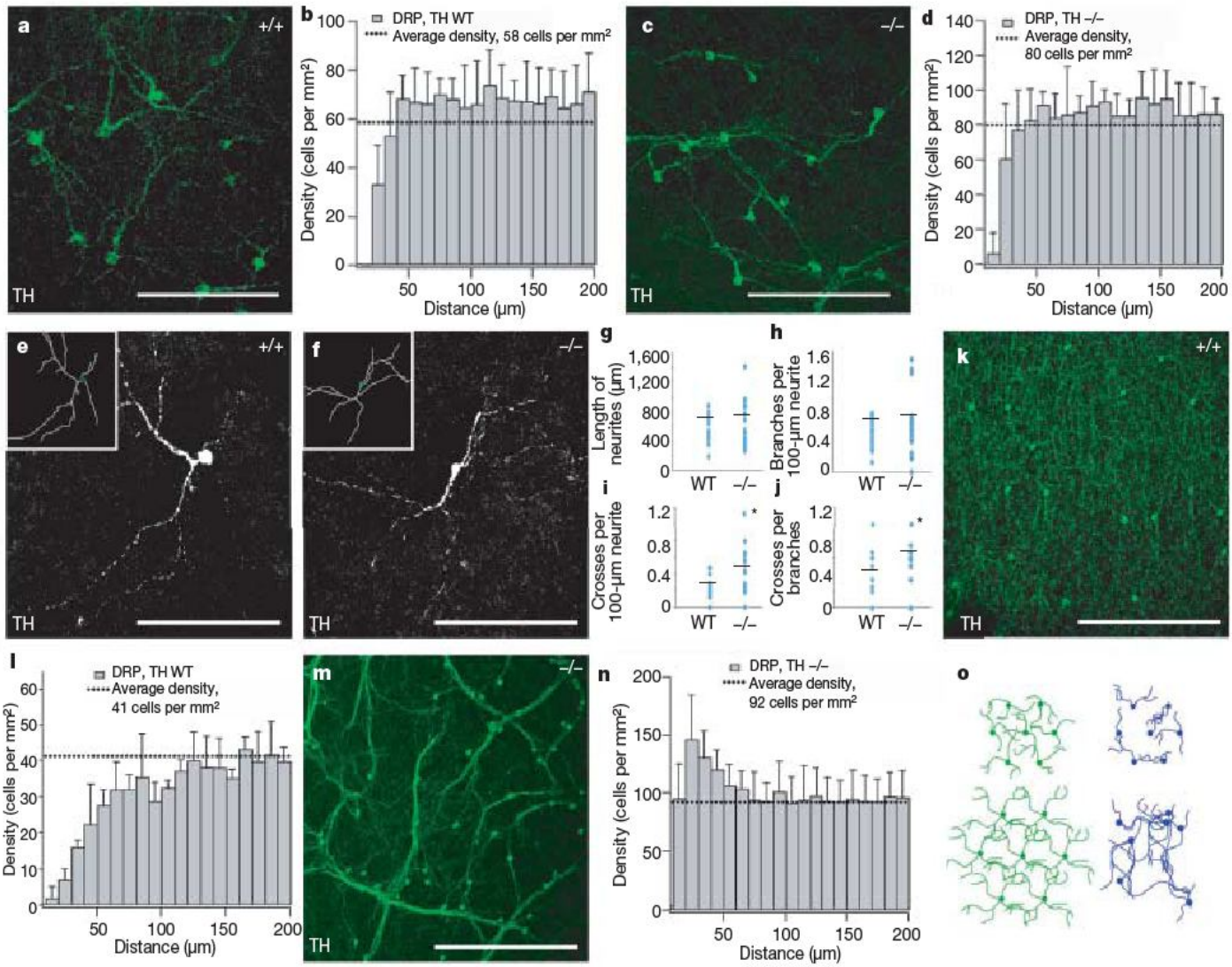

Figure 2 | Arborization and mosaic patterning of dopaminergic amacrine cells in control and *Dscam*$^{-/-}$ retinas. **a**, A wild-type P6 retina stained with anti-TH. Dopaminergic amacrine cell processes have little proximal contact and rarely self-cross. **b**, DRP analysis of cell-body spacing identified an exclusion zone surrounding dopaminergic neurons in wild-type retinas. **c**, A P6 *Dscam*$^{-/-}$ retina stained with anti-TH. Individual dopaminergic amacrine cells frequently self-cross. Error bars in all figures represent s.d. **d**, DRP analysis of *Dscam*$^{-/-}$ dopaminergic amacrine cells indicates normal soma spacing at P6. **e**, **f**, Representative isolated P6 wild-type (**e**) and *Dscam*$^{-/-}$ (**f**) dopaminergic amacrine cells are shown; insets contain traces of the neurites. **g–j**, Analysis of 20 isolated dopaminergic amacrine neurons from either wild-type or *Dscam*$^{-/-}$ retinas. **g**, No significant difference in the total neurite length was observed between wild-type and *Dscam*$^{-/-}$ neurons (*t*-test, $P = 0.55$). **h**, Similarly, no significant difference in neurite branches per unit length was observed ($P = 0.38$). **i**, **j**, A significant increase in the number of self-crossings per length of neurite (**i**) or per number of branches (**j**) was observed for the *Dscam*$^{-/-}$ neurons over controls ($P = 0.003$ and 0.015, respectively). **k–n**, Whole adult (6–8 weeks) wild-type (**k**, **l**) or *Dscam*$^{-/-}$ (**m**, **n**) retinas stained with anti-TH. TH-positive neurite fascicles run throughout the *Dscam*$^{-/-}$ retina (**m**) in contrast to the wild-type retina (**k**), in which neurites arborize evenly. **l**, DRP analysis indicates that dopaminergic amacrine cells in the adult wild-type retina maintain and expand their mosaic spacing and exclusion zones. **n**, DRP analysis of adult *Dscam*$^{-/-}$ retinas indicates aggregation of dopaminergic amacrine neurons. **o**, Schematic of arborization and mosaic formation in wild-type (left) and *Dscam*$^{-/-}$ (right) retinas. At P6, both wild-type and *Dscam*$^{-/-}$ dopaminergic amacrine cells are organized in a mosaic pattern; however, wild-type neurites arborize, whereas *Dscam*$^{-/-}$ neurites self-cross. By P10, wild-type dopaminergic amacrine cells have an increased exclusion zone, whereas *Dscam*$^{-/-}$ dopaminergic amacrine neurons aggregate along bundled fascicles. Scale bars: **a**, **c**, **e**, **f**, 133 μm; **k**, **m**, 388 μm.

from the mutant retinas, consistent with a protein-null mutation (Fig. 1k).

Wild-type dopaminergic amacrine cells were first labelled by anti-TH staining at around P6, when the neurons are still extending processes (Fig. 2a). The spacing of dopaminergic amacrine cell bodies was analysed by density recovery profiling (DRP)—a statistical measure of the cells' spatial autocorrelation, measuring the probability of encountering a homotypic cell body at varied distances from the reference cell. Regularly spaced cells have a zone of exclusion for other homotypic cells, indicated by a below-average cell density at short distances[15] (Fig. 2b). Although their spacing was normal, the *Dscam*$^{-/-}$ dopaminergic amacrine cells showed abnormal morphology at P6 (Fig. 2c, d). Examining individual cell morphologies in 20 isolated dopaminergic amacrine cells in control and *Dscam*$^{-/-}$ retinas revealed defects in arborization in the mutant retina (Fig. 2e, f). Quantification indicated no differences in total length of processes or in the number of branches per unit length, but mutant cells had a significantly larger number of processes that self-crossed

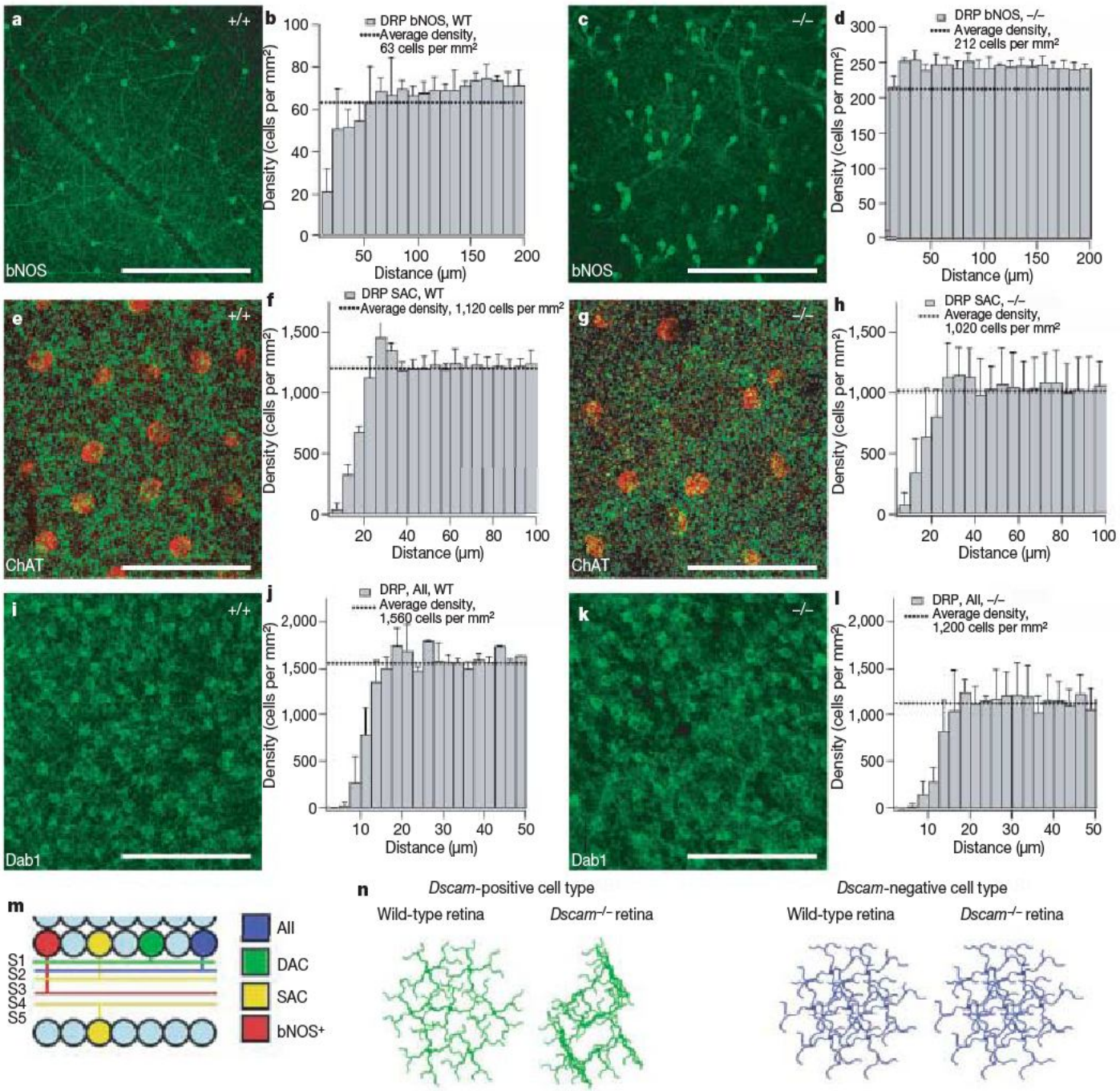

Figure 3 | Morphology and cell body spacing of other amacrine cell types. **a**, **b**, Cell bodies of bNOS-positive cells, which also express *Dscam* in control retinas, are spaced and arborized in a manner similar to dopaminergic amacrine neurons. **c**, **d**, The bNOS-positive amacrine cells show fasciculated processes and randomly spaced cell bodies in *Dscam*$^{-/-}$ retinas. Starburst amacrine cells have an exclusion zone around the cell body and a meshwork of arborized processes in both control (**e**, **f**) and *Dscam*$^{-/-}$ (**g**, **h**) retinas. (Cell bodies are pseudo-colored red to distinguish them from the meshwork of processes in S2 of the IPL (green).) AII amacrine cells are also evenly spaced with very short processes in control (**i**, **j**) and *Dscam*$^{-/-}$ (**k**, **l**) retinas. **m**, Schematic depicting the stratification of bNOS-positive, SAC, DAC and AII neurites in the inner plexiform layer. Dopaminergic amacrine neurites arborize predominantly in S1, AII amacrine neurites arborize in between S1 and S2, bNOS-positive amacrine neurites arborize in S3, and starburst amacrine neurites arborize in S2 (soma in INL) or S4 (soma in RGL). **n**, Schematic summarizing mosaic formation in the *Dscam*$^{-/-}$ retina. DSCAM-positive cell types arborize and maintain mosaics in the wild-type retina, but fasciculate and fail to maintain mosaics in the *Dscam*$^{-/-}$ retina. Cell types in which DSCAM expression was not detected arborize and maintain mosaics in both the wild type and the *Dscam*$^{-/-}$ retina. Scale bars: **a**, **c**, 388 μm; **e**, **g**, 62 μm; **i**, **k**, 123 μm. Different scales are used on the ordinates of DRP graphs for each cell type.

(Fig. 2g–j). This phenotype was more pronounced at P10 (not shown), and eventually large fascicles of TH-positive processes from many cells form in the mutant retina (Supplementary Fig. 6). Dopaminergic processes in the wild-type adult retina form a broad, uniform plexus, and the cell bodies are evenly spaced (Fig. 2k, l). The fasciculated adult *Dscam*$^{-/-}$ dopaminergic amacrine cells had abnormal positioning of their cell bodies, which associated with the fascicles and clumped significantly (Fig. 2m, n and Supplementary Figs 6 and 7).

In the mutant retina, bNOS-positive amacrine cells, which normally express *Dscam*, also had marked differences in the cell morphology, with short bundled processes and randomly spaced cell bodies (Fig. 3a–d). Starburst and AII amacrine populations that do not express *Dscam* were normal in their cell-body distribution (Fig. 3e–l), maintaining an exclusion zone in the control and *Dscam*$^{-/-}$ retinas. The meshwork of processes in the IPL from starburst cells in the inner nuclear layer was preserved in the *Dscam*$^{-/-}$ retinas, with no abnormal fasciculation seen, and the short processes of AII cells were also normal. Cross-sections of control and *Dscam*$^{-/-}$ retinas were also examined, and all cell types reached their appropriate vertical layer in the retina (Supplementary Fig. 8).

In normal retinas, the mosaic spacing of each cell type, such as AII and starburst cells, is independent, allowing dendritic overlap of functionally different cells[16]. If DSCAM-mediated recognition was required for this process, then cell types that express *Dscam* might influence the spacing of each other. We therefore examined the distribution of dopaminergic amacrine and bNOS-positive amacrine cells, relative to each other, in control and mutant retinas (Fig. 4 and Supplementary Fig. 9). These cell bodies were randomly spaced with respect to one another in both genotypes, indicating that DSCAM is necessary for maintaining mosaics in homotypic cells but is not sufficient to confer identifying signals between cell types. Also, despite the fasciculation of dopaminergic and bNOS-positive processes in the *Dscam*$^{-/-}$ retinas, no associations of processes across cell types were seen.

Mammalian DSCAM is therefore required for isoneuronal self-avoidance and for heteroneuronal recognition within a cell type. In the absence of DSCAM, the processes of an individual dopaminergic or bNOS-positive amacrine cell fail to form proper arbors, and instead fasciculate with processes of other cells of the same type, secondarily pulling the cell bodies out of their mosaic position. However, different cell types expressing *Dscam* do not influence each other's spacing. A neurodevelopmental role for vertebrate DSCAM was not previously established beyond an early, non-neuron-specific, developmental phenotype seen in zebrafish[17].

Our results are consistent with previous studies showing that retinal mosaics form very early, before neurons have extensive arbors, and that arborization and mosaic formation are largely independent[18,19]. The normal spacing of cells in the P6 *Dscam*$^{-/-}$ retina indicates that the early stages of mosaic formation are intact. However, DSCAM-mediated heteroneuronal self-avoidance is needed to prevent fasciculation of homotypic cells, which destroys their mosaic pattern.

Decreased cell death may also indirectly contribute to the disruption of mosaics, based on the increase in dopaminergic and bNOS cell densities in the mutant retina. The absolute density of cells that would normally express *Dscam* was increased by ~250% for dopaminergic amacrine cells, and by ~300% for bNOS-positive cells in the mutant retina (DRPs in Figs 2 and 3). In contrast, the densities of the ChAT-positive (starburst) and DAB1-positive (AII) cells were slightly decreased. Dopaminergic amacrine cells rely in part on cell death to sculpt their mosaic pattern[20]. We saw decreased TdT-mediated dUTP nick end labelling (TUNEL)-positive cells in the developing *Dscam*$^{-/-}$ retina, but saw no change in the number of proliferating cells (Supplementary Fig. 10); we also identified that clumped dopaminergic amacrine cells were not clonal, indicating that they are not the result of overproliferation from a progenitor, but consistent with lateral migration of cell bodies (Supplementary Fig. 11). DSCAM-mediated self-recognition may therefore be upstream of cell death in the wild-type retina, whereas non-*Dscam*-expressing cells may use other mechanisms for spacing[21].

The fact that DSCAM mediates both isoneuronal and heteroneuronal self-avoidance in given amacrine cell types is interesting given recent work in *Drosophila*. In flies, *Dscam* is alternatively spliced to generate tens of thousands of protein isoforms[6]. The homophilic interaction of DSCAM is highly isoform-specific, and this specificity is determined by the variable immunoglobulin domains[8,22,23]. Therefore, *Drosophila* neurons use DSCAM for isoneuronal self-avoidance and arborization because each cell recognizes only its own processes, which express the same set of isoforms[8,9,24]. Reducing or eliminating the diversity of DSCAM isoforms perturbs *Drosophila* circuit organization[25,26]. *Dscam2* in *Drosophila* is not extensively alternatively spliced and mediates heteroneuronal cell avoidance in tiling

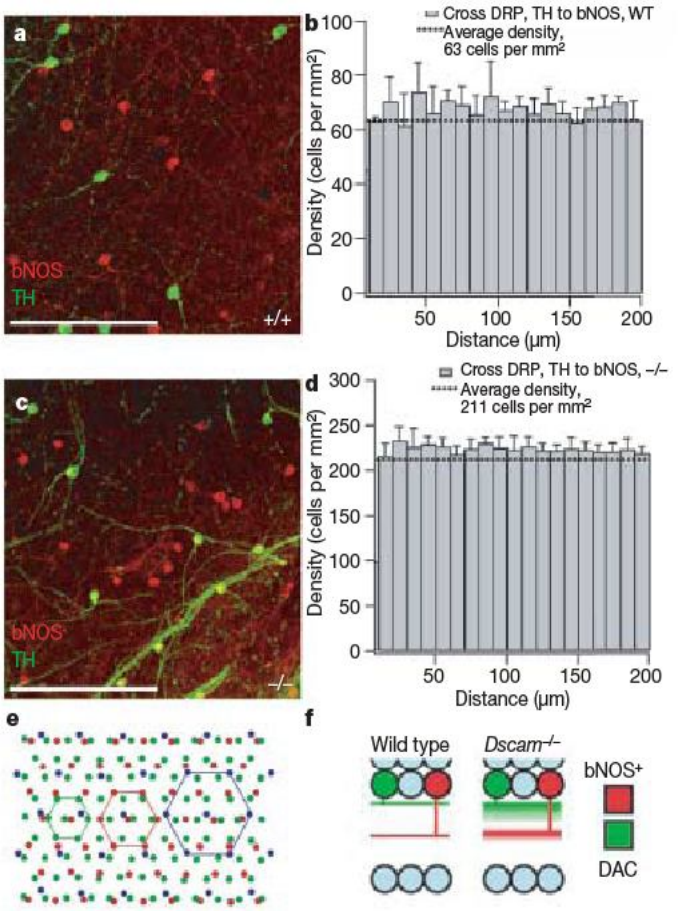

Figure 4 | Co-existence of dopaminergic and bNOS-positive amacrine cells. **a**, In wild-type retinas double-labelled for two *Dscam*-expressing cell populations (dopaminergic amacrine and bNOS-positive), the cell bodies and processes appear independent. **b**, This was confirmed by cross-DRP analyses comparing dopaminergic to bNOS-positive cells, which indicated no difference from average density (random spacing). **c**, In *Dscam*$^{-/-}$ mutant retinas, the fasciculation and cell-body-spacing defects are evident, but the spacing of cells remains independent and they do not aggregate, as indicated by cross-DRP analyses (**d**). In addition, the fascicles of processes also remain independent. **e**, Schematic depicting the independence of retinal neuron mosaic formation. Retinal neurons form organized mosaics that are independent of the spacing of other cell types. **f**, Schematic depicting dopaminergic amacrine and bNOS-positive neuron arborization in the wild-type and *Dscam*$^{-/-}$ retina. Wild-type dopaminergic amacrine neurons arborize in S1, whereas bNOS-positive neurons arborize in S3. In the *Dscam*$^{-/-}$ retina, the arborization of dopaminergic amacrine and bNOS-positive neurites becomes diffuse but, although neurites of both cell types fasciculate with homotypic neurites (dopaminergic amacrine with dopaminergic amacrine or bNOS-positive with bNOS-positive), they do not fasciculate with heterotypic neurites (dopaminergic amacrine with bNOS-positive). Scale bars in **a** and **c** represent 240 μm.

the axons of L1 neurons in the medulla of the *Drosophila* visual system[7]. Mouse DSCAM functions in a highly analogous way, promoting self-avoidance for both arborization (isoneuronal) and the prevention of fasciculation (heteroneuronal). Although the vertebrate genes are not extensively alternatively spliced, they do undergo homophilic and paralogue-specific binding[5,27,28]. The coexistence of *Dscam*-expressing amacrine cell populations suggests that stratification in different IPL layers may allow reuse of DSCAM as a recognition signal. Alternatively, additional co-signals may distinguish distinct cell populations, consistent with the continued lack of interaction between bNOS and dopaminergic cells in the $Dscam^{-/-}$ retina. Additional complexity for self-recognition could also be introduced by other *Dscam* gene family members, such as *Dscam-like 1* (*Dscaml1*), which is expressed in a similar but discrete population of retinal neurons[27,28] (P.G.F. and R.W.B., unpublished) or the highly homologous Sidekick proteins[29].

METHODS SUMMARY

Genetics and *Dscam* analysis. The *Dscam* mutation was mapped by breeding to C57BL6/J and establishing linkage in F2 mice with polymorphic markers, and the mutation was identified by positional cloning approaches. Mice were genotyped by PCR from genomic DNA spanning the 38-bp deletion (*Dscam* forward, CTTTGCGCGTTATGATCCT; *Dscam* reverse, GTGGTGTCGATACTGATG). Control mice were littermates of the $Dscam^{-/-}$ animals to control for genetic background effects and age.

Immunocytochemistry. Whole-mount dissected retinas or cryostat sections were stained using standard immunofluorescence protocols. Antibody sources are listed in the Methods. Fluorescence images were collected using a Leica SP5 confocal microscope or a Nikon epifluorescence microscope with a digital camera.

***In situ* hybridization.** *In situ* hybridization was performed using digoxygenin- and fluorescein isothiocyanate (FITC)-labelled riboprobes, detected by HRP-conjugated secondary antibodies and TSA-Plus fluorescent substrates (Perkin Elmer).

Analysis of mosaics. The spacing of cell bodies was analysed using DRP as described previously, with the modification that the closest bin in the analysis was corrected to account for the diameter of the cell body[15,16].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins

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Light and gibberellins (GAs) mediate many essential and partially overlapping plant developmental processes. DELLA proteins are GA-signalling repressors that block GA-induced development[1]. GA induces degradation of DELLA proteins via the ubiquitin/proteasome pathway[2], but light promotes accumulation of DELLA proteins by reducing GA levels[3]. It was proposed that DELLA proteins restrain plant growth largely through their effect on gene expression[4,5]. However, the precise mechanism of their function in coordinating GA signalling and gene expression remains unknown. Here we characterize a nuclear protein interaction cascade mediating transduction of GA signals to the activity regulation of a light-responsive transcription factor. In the absence of GA, nuclear-localized DELLA proteins accumulate to higher levels, interact with phytochrome-interacting factor 3 (PIF3, a bHLH-type transcription factor) and prevent PIF3 from binding to its target gene promoters and regulating gene expression, and therefore abrogate PIF3-mediated light control of hypocotyl elongation. In the presence of GA, GID1 proteins (GA receptors) elevate their direct interaction with DELLA proteins in the nucleus, trigger DELLA protein's ubiquitination and proteasome-mediated degradation, and thus release PIF3 from the negative effect of DELLA proteins.

Light and GA interact during *Arabidopsis thaliana* seedling development, regulating hypocotyl elongation, cotyledon opening and light-responsive gene expression; their pathways seem to converge at regulation of the abundance of DELLA proteins (GA pathway repressors)[3,6]. *Arabidopsis* has five DELLA proteins—RGA, GAI, RGL1, RGL2 and RGL3—defined by their unique DELLA domain and a conserved GRAS domain[4]. To analyse them *in vivo*, we raised antibodies against endogenous RGA and generated transgenic *Arabidopsis* expressing each of the five DELLA proteins with tandem affinity purification (TAP) tags (Supplementary Fig. 1). The response of DELLA protein levels to exogenously applied GA_3 (an active form of GA) or PAC (paclobutrazol, a GA biosynthesis inhibitor) was examined. We found that one-hour-long GA treatment eliminates the majority of DELLA proteins, and this GA effect can be largely prevented by 100 μM MG132 (a 26S proteasome-specific inhibitor). PAC, on the other hand, promotes over-accumulation of DELLA proteins (Fig. 1). These results show for the first time in *Arabidopsis* that all the DELLA proteins are under negative control by GA and the proteasome. Next, we generated lines expressing TAP-tagged RGAΔ17 and GAIΔ17, which lack a 17 amino acid motif within the DELLA domain that is required for GA-induced degradation[7,8]. As expected, TAP–RGAΔ17 and TAP–GAIΔ17 are completely resistant to GA and accumulate at higher levels than wild-type proteins, which cannot be further increased by PAC (Fig. 1, and

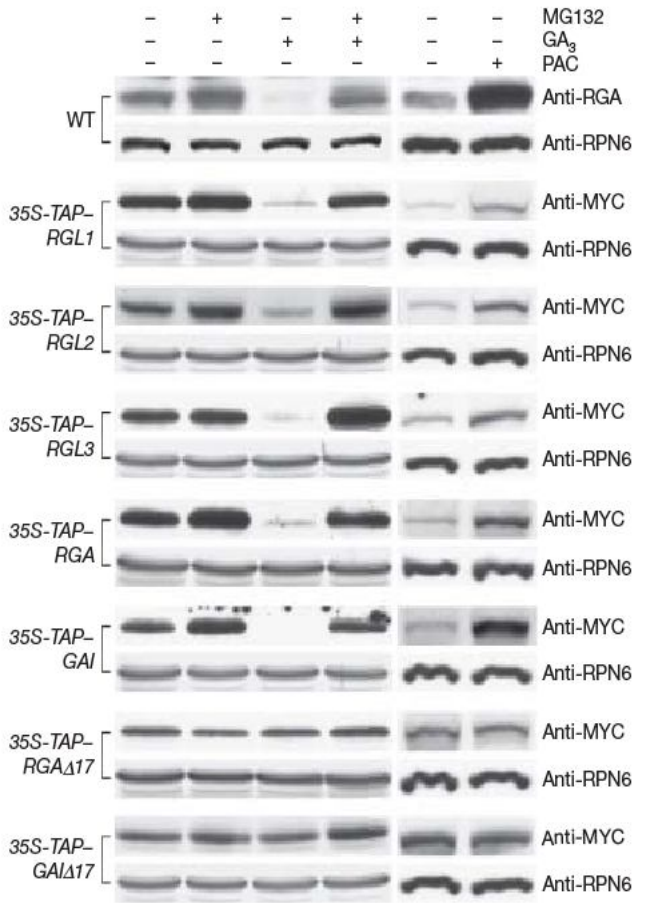

Figure 1 | Effect of GA_3, MG132 and PAC on DELLA protein abundance. Immunoblot analysis of RGA (by anti-RGA antibody) and TAP-DELLA proteins (by anti-MYC antibody) in various light-grown *Arabidopsis* seedlings (genotypes labelled to the left of each panel) treated with different combinations of GA_3, MG132 and PAC. Panels on the left (four lanes) and panels on the right (two lanes) are from two independent experiments using different protein gel systems. RPN6 immunoblotting (by anti-RPN6 antibody) is used as a loading control. WT, wild type.

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Supplementary Fig. 1b). *Arabidopsis* plants that overexpress these proteins show a dominant dwarf phenotype, reflecting enhanced DELLA activity (Supplementary Fig. 2), which also suggests that TAP–DELLA proteins retain normal DELLA function.

Inhibition of hypocotyl elongation, an important characteristic of photomorphogenesis, is shown to be repressed by GA in the dark and promoted by DELLA proteins in the light[3,6]. We further examined the possible mechanism of DELLA proteins in regulating photomorphogenesis. *Arabidopsis* seedlings have longer hypocotyls on GA-containing medium, whereas PAC dramatically inhibits the elongation of hypocotyls (Fig. 2a, b). Furthermore, the GA effect is more drastic in red light than in dark (Fig. 2b), consistent with the notion that the endogenous GA level is higher in dark-grown seedlings. In addition, *35S-TAP–RGAΔ17* and *35S-TAP-GAIΔ17* plants have much shorter hypocotyls than wild type, which cannot be rescued by GA. On the contrary, the hypocotyl of *rga-24 gai-t6* double mutants is longer than that of wild type, and is only partially inhibited by PAC. In a pentuple mutant (*della*) of all five *DELLA* genes, the hypocotyl length is comparable to that of GA-treated wild type, and PAC has no significant effect (Fig. 2a, b). Therefore, we reasoned that GA controls hypocotyl growth and affects photomorphogenesis status, mainly by regulating DELLA protein abundance.

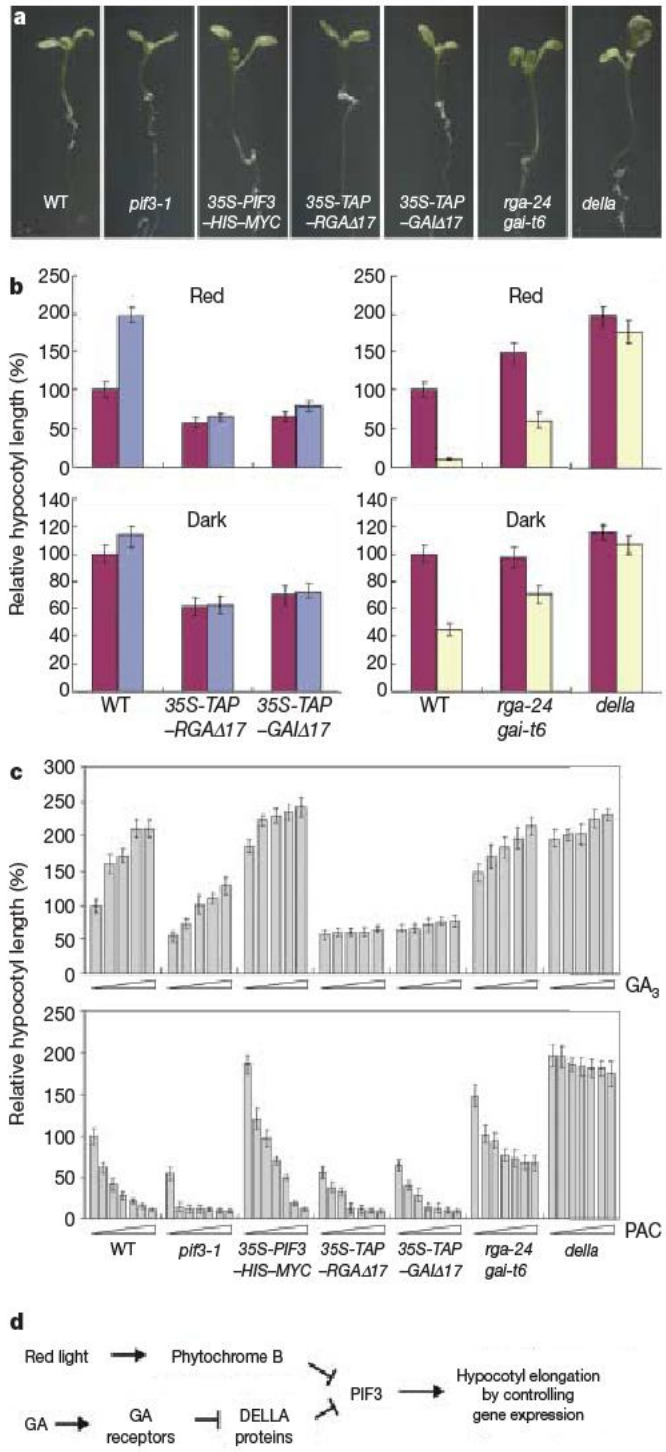

Figure 2 | DELLA proteins and PIF3 have opposite roles in regulating *Arabidopsis* hypocotyl elongation. **a**, Images of red-light-grown seedlings. **b**, Hypocotyl length measurement (mean ± s.d.) of untreated seedlings (red), or seedlings treated with 10 μM GA_3 (blue) or 1 μM PAC (yellow). **c**, Hypocotyl length measurement (mean ± s.d.) of red-light-grown seedlings treated with increasing amounts of GA_3 or PAC (see Methods). The concentrations of GA_3 used are 0, 0.5 μM, 1 μM, 2 μM and 5 μM (from left to right). The concentrations of PAC were 0, 0.01 μM, 0.02 μM, 0.05 μM, 0.1 μM, 0.2 μM and 0.5 μM (from left to right). In **b** and **c**, hypocotyl length of untreated wild-type seedlings is set to 100%. **d**, Simplified diagram depicting the genetic interaction of light and GA in the control of hypocotyl elongation by PIF3 and DELLA proteins. *della*, *rga-t2 gai-t6 rgl1-1 rgl2-1 rgl3-1*.

DELLA proteins are proposed to be transcription factors[4], and are required to localize to the nucleus for their function[9,10]. Genomic studies have revealed a number of GA-responsive genes that are regulated by *DELLA* genes[5]. However, using the chromatin immunoprecipitation (ChIP) technique in *35S-TAP–DELLA* lines, we were unable to observe specific binding of DELLA proteins to any of the 38 GA-responsive gene promoters tested (Supplementary Table 1). Thus, we hypothesize that DELLA proteins might regulate gene expression indirectly by controlling transcription factors. Because light and DELLA proteins both regulate hypocotyl growth, it seems possible that one, or more, of the well-known photomorphogenesis-related transcription factors might be a target of DELLA proteins. Among them, PIF3 is a good candidate, because it promotes hypocotyl elongation in red light[11]—the opposite of DELLA's function (Fig. 2a). Moreover, PIF3 has DNA-binding activity[12], interacts with the active form of phytochrome B (phyB)[13,14], and is negatively regulated by phytochrome through the ubiquitin/proteasome pathway[15–17], indicating it mediates signalling between light and gene expression. We observed that the *pif3-1* mutant has a short hypocotyl, and is partially resistant to GA and hypersensitive to PAC, mimicking *35S-TAP–RGAΔ17* and *35S-TAP–GAIΔ17* plants, whereas the PIF3 overexpression line shows a long hypocotyl and is hyposensitive to PAC, in a similar manner to GA-treated plants and *rga-24 gai-t6* and *della* mutants (Fig. 2c). These results imply that DELLA proteins may negatively regulate PIF3 in the control of hypocotyl elongation, representing a convergent point of light and GA pathways (Fig. 2d).

This regulation is probably mediated through physical interaction between PIF3 and DELLA proteins, as suggested by yeast two-hybrid and *in vitro* pull-down assays (Fig. 3a, d, and Supplementary Fig. 3). Moreover, bimolecular fluorescence complementation (BiFC) analysis detects direct RGA–PIF3 interaction in the nuclei of living plant cells (Fig. 3b). We further investigated this interaction using an immunoprecipitation approach. As shown in Fig. 3c, interaction between RGA and PIF3 is observed in dark-grown seedlings, in which PIF3 protein accumulates to reasonable abundance[15–17]. The interaction is also detectable in red light, when light-induced proteasomal degradation of PIF3 (refs 15–17) is blocked. The interaction is dependent on RGA abundance, such that PAC increases RGA–PIF3 interaction, whereas GA abolishes RGA accumulation and thus PIF3 is released. Importantly, under the condition that RGA–PIF3 interaction is enhanced, PIF3's effect on hypocotyl growth is largely impaired, and vice versa (Figs 2c, 3c), indicating that RGA-bound PIF3 has reduced activity. We tested whether DELLA proteins influence the previously reported interaction between phytochrome and PIF3 (refs 13, 14) by analysing the formation of nuclear speckles containing both phyB and PIF3 (ref. 15). Evidently, phyB–PIF3 interaction is essentially not affected by altering DELLA protein abundance (Supplementary Fig. 4). Therefore, DELLA protein binding most probably affects PIF3's transcription-regulation activity towards its target genes.

This notion is supported by the observation that the RGA–PIF3 interaction *in vitro* is specifically inhibited by pre-incubating PIF3 with its cognate binding site, a G-box-containing DNA probe[12]

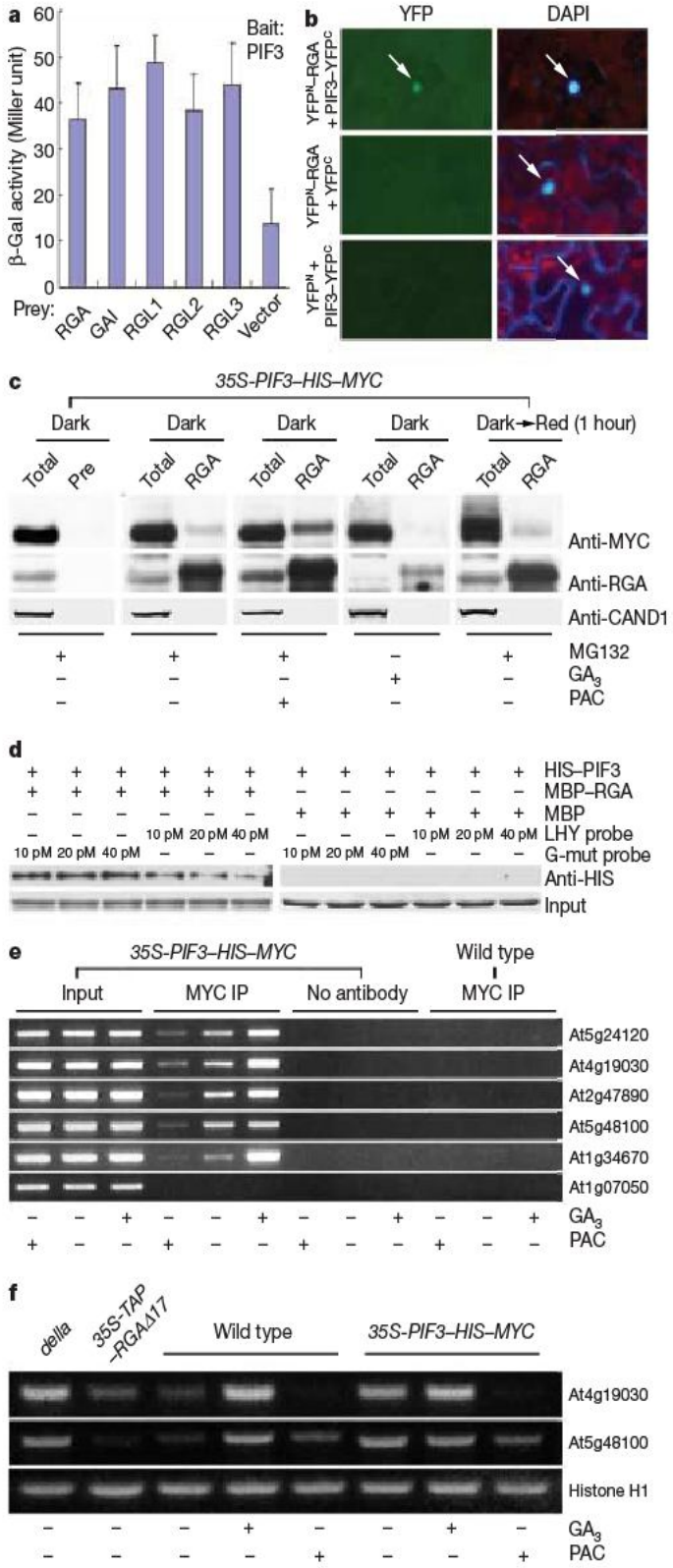

Figure 3 | DELLA proteins bind PIF3 and inhibit PIF3 activity towards its target genes. **a**, β-galactosidase activities from yeast two-hybrid assays (mean ± s.d.). **b**, BiFC analysis of RGA and PIF3. The positions of nuclei are indicated by arrows. **c**, Co-immunoprecipitation of RGA with PIF3 in *35S-PIF3–His–MYC* seedlings. 'RGA' and 'Pre' indicate immunoprecipitation by anti-RGA antibody and pre-immune sera, respectively. **d**, Pull-down assays between His–PIF3 and MBP–RGA. The precipitated His–PIF3 was detected by anti-His antibody. MBP–RGA and MBP inputs were stained by Coomassie blue. **e**, ChIP–PCR analyses in dark-grown seedlings. **f**, Semi-quantitative RT–PCR analyses in dark-grown seedlings. Total, total protein extracts; IP, immunoprecipitation.

(LHY; Fig. 3d, and Supplementary Fig. 3), which provides evidence that RGA–PIF3 and PIF3–DNA bindings are antagonistic. To test this *in vivo*, we selected five putative PIF3 target genes by analysing the published literature as well as taking into account results we obtained from a ChIP microarray analysis focused on PIF3, using a recently reported method[18]. By ChIP–PCR, we confirmed that these five promoters are bound by PIF3 as expected. In addition, we found that when DELLA protein level is increased by PAC, PIF3–promoter binding is severely reduced. On the other hand, removing DELLA proteins by GA treatment generally leads to enhanced occupancy of PIF3 on the promoters (Fig. 3e). We also noticed that, whereas GA and PAC do not significantly affect nuclear PIF3–MYC protein levels, they have slightly opposite effects on PIF3–MYC immunoprecipitation (Supplementary Fig. 5), which might be due to higher affinity of MYC antibody towards free PIF3–MYC than RGA-bound PIF3–MYC. Among the PIF3 target genes, At5g2120, At4g19030, At2g47890 and At1g34670 show light-responsive expression[19]. Interestingly, differential expression of At5g24120, At4g19030 and At2g47890 have also been reported in genomic studies focused on gene expression regulation by GA, *PIF3* or *DELLA* genes[5,20,21]. Subsequently, we used PCR with reverse transcription (RT–PCR) to check whether PIF3–promoter binding indeed affects gene expression. As shown in Fig. 3f, overexpressing PIF3 and reducing DELLA protein abundance (*della* mutant or GA treatment) have similar effect on the expression of two representative PIF3 target genes, whereas increasing DELLA protein abundance (overexpressing RGAΔ17 or PAC treatment) has the opposite effect. Overall, we demonstrate that DELLA proteins antagonize PIF3 function by protein–protein interaction and sequestration, which at least partly explains their effect on gene expression and the coordinated control of hypocotyl growth by light and GA.

We next examined how the GA signal is relayed to affect DELLA protein abundance and thus DELLA–PIF3 interaction. Recently, GID1 proteins have been shown to act as nuclear GA receptors[22–25]. Through isolating and analysing *Arabidopsis gid1* mutants, we obtained results that are consistent with those reports[24,25], suggesting that GID1s are required for normal GA signalling and participate in light-induced development, possibly by inducing DELLA protein degradation (Supplementary Note 1 and Supplementary Fig. 6). We also confirmed the reported GA-dependent GID1–DELLA interaction[22–25], which requires the 17 amino acid motif within the DELLA domain, in yeast two-hybrid assays (Fig. 4a, b, and Supplementary Fig. 7). In addition, BiFC analysis of GID1c and RGA demonstrates their direct interaction in the nuclei of living plant cells (Fig. 4c). To test the effect of GA on GID1–DELLA interaction *in planta*, we used transgenic *Arabidopsis* expressing each of the three GID1 proteins with YFP or epitope tags for immunoprecipitation analyses. As shown in Fig. 4d, interaction of GID1a with each of the five DELLA proteins is detectable, and is greatly enhanced by GA. Furthermore, with MG132 treatment, immunoprecipitated DELLA proteins contain high-molecular-weight protein species, which react specifically with anti-ubiquitin antibody. Increasing amounts of these DELLA proteins that are predicted to be multi-ubiquitinated can be detected after GA treatment (Fig. 4d, e). On the other hand, GAIΔ17 shows no detectable interaction with GID1a and a nearly complete loss of multi-ubiquitination (Fig. 4d), suggesting that interaction with GID1s is essential for the multi-ubiquitination of DELLA proteins. We extended the interaction study to all three GID1 proteins, and the co-immunoprecipitation of each GID1 with endogenous RGA was detected (Fig. 4f–h). In these experiments, we lowered MG132 concentration (40 μM) and lengthened GA treatment time (two hours) to only partially inhibit the proteasome and allow a significant portion of RGA to be degraded. Nevertheless, GA-treated seedlings still have more GID1-bound RGA (Fig. 4f–h), implying that GID1–DELLA may be recognized by the ubiquitin/proteasome system as a heteromeric complex, and somehow DELLA proteins are preferentially ubiquitinated and degraded. This is supported by the

observation that GID1a enhances RGA–SLY1 (E3 ubiquitin ligase subunit) interaction in the presence of GA[24]. Consistent with previous results in yeast[22], GID1b binds more RGA in untreated seedlings, and even with PAC treatment a small amount of GID1b-bound RGA can be detected (Fig. 4f-h). This implies a possible GA-independent pathway for GID1b to target DELLA proteins, which might be critical to keep DELLA proteins in check when the GA level is low.

The results reported here support a conclusion that GA signalling is initiated when GA molecules, the biosynthesis of which is induced by light[3], are sensed and bound by nuclear GID1 proteins. Then, GA-charged GID1s interact with DELLA proteins in the nucleus and target them for proteasomal degradation. When DELLA protein abundance is reduced, their interactive partners, for example, light-responsive and phytochrome-interacting transcription factors such as PIF3, are released from sequestration, and bind to their target promoters and regulate gene expression (Fig. 4i). PIF3 belongs to a transcription factor family defined by a conserved bHLH (basic-helix–loop–helix) domain, which has implicated function in DNA binding and dimerization[26]. In a similar way to PIF3, PIF4 (another phytochrome-interacting bHLH transcription factor) is also negatively regulated by DELLA proteins[27]. Moreover, DELLA proteins are shown to interact with the DNA-binding bHLH domain[27], consistent with our observation that RGA–PIF3 and PIF3–promoter interactions are mutually exclusive (Fig. 3). Two other bHLH proteins, PIL5 and SPT, are also involved in light and GA signalling, and have PIF3-like roles in hypocotyl growth[28,29], making them potential targets of DELLA proteins as well. Collectively, it is highly plausible that,

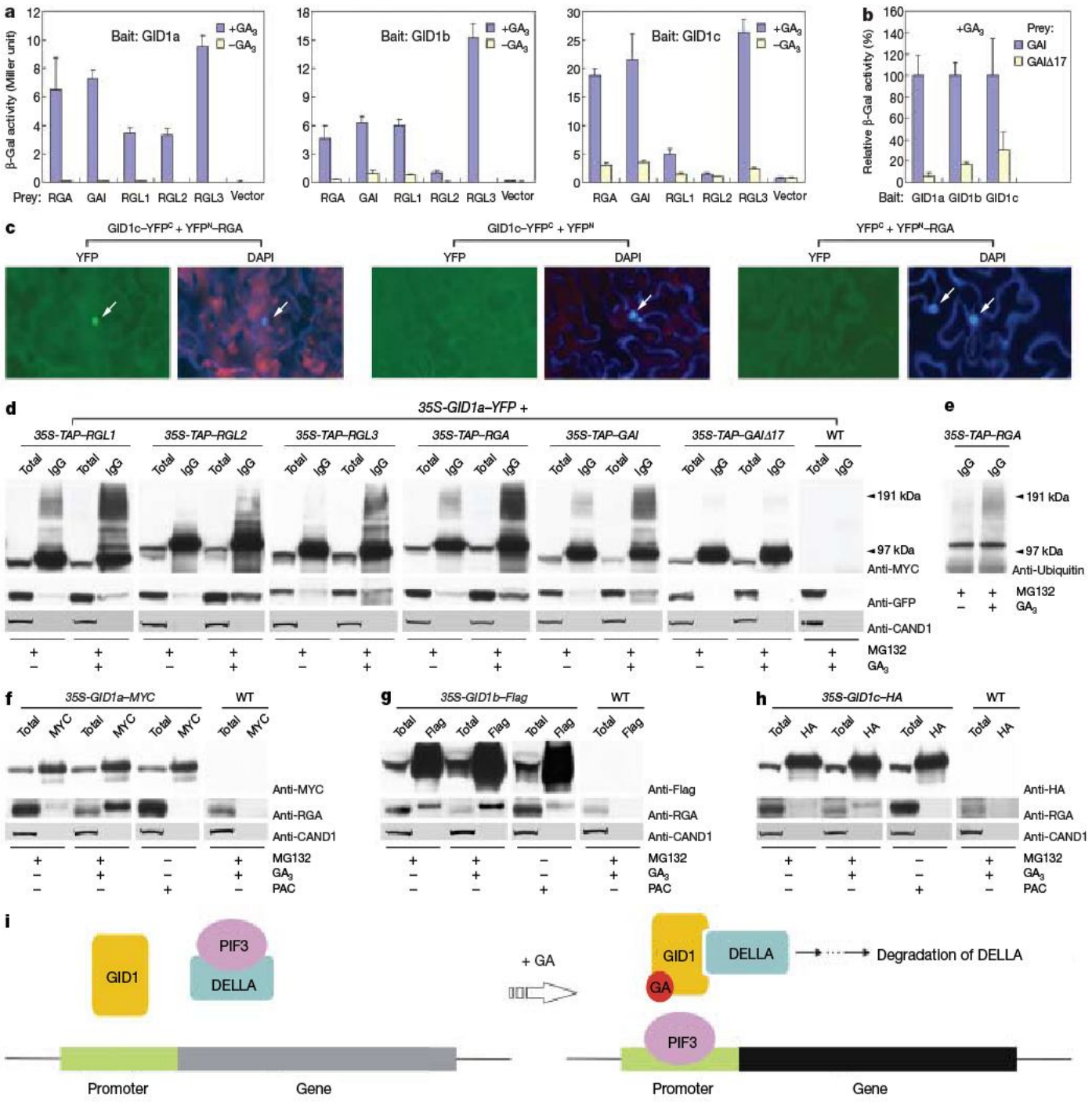

Figure 4 | GA-dependent interaction between GID1s and DELLA proteins. **a**, **b**, β-galactosidase activities from yeast two-hybrid assays (mean ± s.d.). In **b**, β-galactosidase activities from GAI–GID1 interactions are set to 100%. **c**, BiFC analysis of GID1c and RGA. The positions of nuclei are indicated by arrows. **d**, TAP-DELLA proteins interact with GID1a–YFP. **e**, Detection of multi-ubiquitinated TAP-RGA. In **d**, **e**, 'IgG' indicates immunoprecipitation by IgG-conjugated beads. **f**–**h**, RGA interacts with GID1a–MYC (**f**), GID1b–Flag (**g**), and GID1c–HA(haemagglutinin) (**h**). 'MYC', 'Flag' and 'HA' indicate immunoprecipitation by respective antibodies. **i**, A working model of the nuclear protein interaction cascade in GA signalling.

through modulating multiple phytochrome-interacting transcription factors, DELLA proteins play a key part in integrating the regulatory effect of light and GA on gene expression and plant development.

METHODS SUMMARY

The procedures for *Arabidopsis* plant growth, yeast two-hybrid analyses, protein and chromatin immunoprecipitations, and subcellular localization studies are described previously[15,18,30]. CAND1 is used as a negative control in protein immunoprecipitation experiments. Histone H1 is used as an internal control in RT–PCR. Primers used in ChIP–PCR and RT–PCR are listed in Supplementary Tables 1 and 2. MG132 treatment is carried out by vacuum infiltration. GA and PAC treatments are carried out by either applying GA_3 to the seedlings or supplementing plant growth medium with GA_3 or PAC. BiFC experiments are carried out between transiently expressed *Arabidopsis* proteins in tobacco leaves. *In vitro* pull-down assays are performed using recombinant proteins purified from bacteria, in the presence of either a canonical G-box containing DNA probe (LHY) or a mutant G-box-containing DNA probe (G-mut).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions X.W.D. conceived the project, and S.F. and X.W.D. together designed the experiments. L.-M.F. designed some of the experiments. S.F. and G.G. performed chromatin immunoprecipitation. C.M. and G.G. analysed the *gid1* mutants and performed RT–PCR. Y.W. made the anti-RGA antibody. Y.W., L.C., F.W. and L.Y. performed the yeast two-hybrid analyses. J.Z. and F.W. performed *in vitro* pull-down assays. C.M. and J.M.I.-P. performed the BiFC experiments. S.K. and E.S. performed phyB and PIF3 subcellular localization studies. X.F. provided the *rga-24 gai-t6* double and *della* pentuple mutants. S.F. performed all other experiments. S.F. and X.W.D. wrote the manuscript.

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LETTERS

A molecular framework for light and gibberellin control of cell elongation

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Cell elongation during seedling development is antagonistically regulated by light and gibberellins (GAs)[1,2]. Light induces photomorphogenesis, leading to inhibition of hypocotyl growth, whereas GAs promote etiolated growth, characterized by increased hypocotyl elongation. The mechanism underlying this antagonistic interaction remains unclear. Here we report on the central role of the *Arabidopsis thaliana* nuclear transcription factor PIF4 (encoded by *PHYTOCHROME INTERACTING FACTOR 4*)[3] in the positive control of genes mediating cell elongation and show that this factor is negatively regulated by the light photoreceptor phyB (ref. 4) and by DELLA proteins that have a key repressor function in GA signalling[5]. Our results demonstrate that PIF4 is destabilized by phyB in the light and that DELLAs block PIF4 transcriptional activity by binding the DNA-recognition domain of this factor. We show that GAs abrogate such repression by promoting DELLA destabilization, and therefore cause a concomitant accumulation of free PIF4 in the nucleus. Consistent with this model, intermediate hypocotyl lengths were observed in transgenic plants over-accumulating both DELLAs and PIF4. Destabilization of this factor by phyB, together with its inactivation by DELLAs, constitutes a protein interaction framework that explains how plants integrate both light and GA signals to optimize growth and development in response to changing environments.

Seedlings undergo alternative developmental programmes depending on whether they are germinated in the dark or in the light. Dark-grown seedlings exhibit etiolated growth, characterized by long hypocotyls, small and closed cotyledons with undifferentiated chloroplasts, and the repression of light-regulated genes[1]. During photomorphogenesis, light inhibits hypocotyl growth and promotes cotyledon opening and expansion, chloroplast differentiation and the activation of light-regulated genes. phyB is the main photoreceptor mediating de-etiolation in red light[4,6]. Absorption of red light converts this photoreceptor into a Pfr active form that is translocated into the nucleus[7,8]; Pfr interacts there with members of the bHLH family of phytochrome-interacting factors (PIFs), involved in modulation of light-regulated genes with a role in photomorphogenesis[1,4].

Gibberellins (GAs) exert an opposite effect to light on photomorphogenesis[2]. GAs promote etiolated growth, whereas GA-deficiency induces a partially de-etiolated phenotype in the dark, which is reverted by a lack of DELLA function[2,9]. DELLAs function as key repressors of GA-responsive growth, by inhibiting GA-regulated gene expression[5]. These repressors accumulate in the nucleus and are rapidly degraded in response to GA[10,11]. In *Arabidopsis*, RGA (encoded by *repressor of ga1-3*) and GAI (encoded by *GA insensitive*) are the main repressors controlling hypocotyl growth and stem elongation[12,13]. Mutations within the DELLA domain render these proteins resistant to degradation, and result in a GA-insensitive dwarf phenotype[12,14]. This domain binds the GA receptor GID1 (ref. 15) in a GA-dependent manner, which promotes interaction with the F-box protein SLEEPY1 (SLY1) and polyubiquitination of these proteins by the $SCF^{SLY1/GID2}$ ligase complex, thereby signalling their degradation by the 26S proteasome pathway[16,17].

The functional mechanism by which DELLAs regulate gene expression and promote photomorphogenesis remains unclear. Attempts to demonstrate direct DNA-binding ability of DELLAs have been unsuccessful, indicating that these repressors might exert their negative regulatory function through protein–protein interaction with other transcription factors. Here we report on the interaction of DELLAs with the PIF4 and PIF3 bHLH factors using a yeast two-hybrid-based screen (Fig. 1a and Supplementary Fig. 1), and provide evidence for a crucial role of these factors in the integration of both GA and light signals to modulate hypocotyl growth.

Pull-down assays using a purified glutathione *S*-transferase (GST)–RGA protein confirmed interaction of DELLAs with the PIF4 and PIF3 factors and showed an increased affinity for PIF4 binding (Fig. 1b). Bimolecular fluorescence complementation (BiFC) assays in *Nicotiana benthamiana* leaves demonstrated interaction of these proteins in living plant cells (Fig. 1c). In addition, co-immunoprecipitation studies using transgenic green fluorescent protein (GFP)–RGA lines[10] further corroborated this interaction (Fig. 1d). Co-immunoprecipitation with an anti-GFP antibody and subsequent detection with an antibody raised against the PIF4 protein showed that binding to the RGA repressor is more efficient in seedlings treated with the inhibitor of GA biosynthesis paclobutrazol (PAC), which induces RGA accumulation, and also in seedlings exposed to dark (see Fig. 1d). Treatment with GA promotes RGA degradation and abolishes this interaction (Fig. 1d).

The *pif4* mutant has short hypocotyls in red and white light, whereas PIF4 overexpressors (35S-PIF4) show a long-hypocotyl phenotype that resembles the *phyB* mutants[3]. We also demonstrate that these plants have an altered response to PAC and GA treatments, indicative of a PIF4 role in GA-induced hypocotyl growth control. Response to increasing concentrations of PAC was reduced in *phyB* and *35S-PIF4* (in an *slr2* background) seedlings, whereas *pif4* seedlings showed a hypersensitive response to this inhibitor (Fig. 2a, c). GA application, in turn, induced an exaggerated elongation response in *phyB* and *35S-PIF4* seedlings, whereas *pif4* was partially insensitive to this treatment (Fig. 2b, c). These results thus point to a possible

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function of this transcription factor as an integration node for both the light and GA pathways.

Deletion studies revealed that the PIF4 bHLH DNA-recognition domain is responsible for interaction of this factor with RGA (Supplementary Fig. 2a). In addition, we determined that the first conserved heptad leucine repeat in the RGA protein mediates interaction of this repressor with PIF4 (Supplementary Fig. 2b). Of note, this heptad repeat region is highly conserved in all members of the DELLA family and an interaction between PIF4 with the GAI, RGL1 or RGL3 repressors is also observed in yeast cells (Supplementary Fig. 3). Identification of the PIF4 bHLH domain as the domain mediating interaction with the DELLAs raised the possibility that these repressors may block PIF4 DNA-binding ability. To test this possibility, we performed transient expression assays, using a *GUS* (β-glucuronidase gene) fusion to the *LTP3* promoter (At5g59320) as a reporter for PIF4 transcriptional activity. Effector constructs for the PIF4 and RGA proteins, and for deletion derivatives of the DELLA repressor resistant to GA destabilization (ΔRGA) or those unable to interact with PIF4 (del1RGA), were expressed under control of the 2× *CaMV 35S* promoter and co-bombarded together with the reporter construct into *Arabidopsis thaliana* cells. As seen in Fig. 2d, expression of PIF4 resulted in a 2.6-fold stimulation of the *LTP3* reporter activity, providing evidence for a positive regulatory activity of this factor. Co-expression of PIF4 and the RGA or ΔRGA proteins strongly repressed *LTP3* expression, demonstrating that these repressors block PIF4 transcriptional activity. More importantly, treatment with GA suppressed the inhibitory effect of RGA by triggering degradation of the protein. However, the stable ΔRGA protein lacking the DELLA domain was unresponsive to this treatment (Fig. 2d). Co-expression of del1RGA, in turn, did not suppress PIF4 transcriptional activity (Fig. 2d), demonstrating that the observed repression is dependent on RGA–PIF4 interaction. Hence, these results are congruent with a positive regulatory function of PIF4 in cell elongation, and with DELLAs repressing PIF4 activity by forming an inactive complex with this factor.

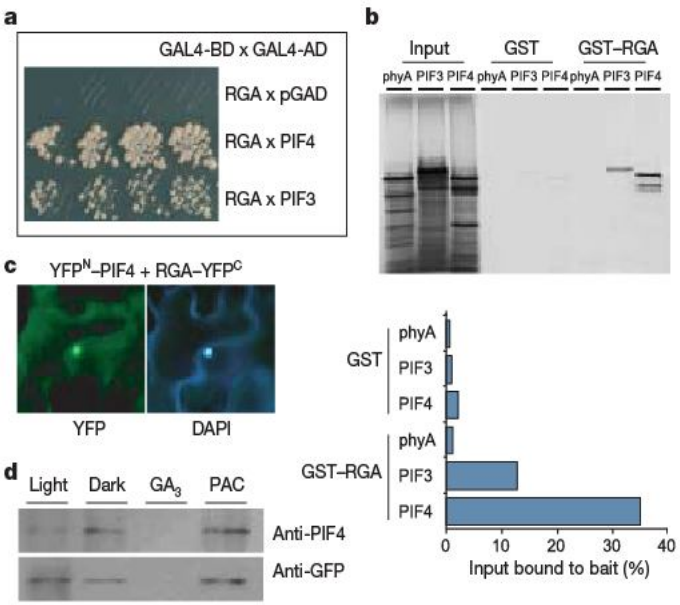

Figure 1 | DELLA–PIF4 interaction in yeast two-hybrid and pull-down assays and interaction of these proteins in *Nicotiana benthamiana* leaves and *Arabidopsis thaliana* seedlings. **a**, Interaction between DELLA and the PIF4 and PIF3 bHLH factors in −His −Ade plates. BD, binding domain; AD, activation domain. **b**, Pull-down assays showing the interaction between RGA and the PIF3 and PIF4 factors. Quantification of the radio-labelled pulled down phyA, PIF3 and PIF4 proteins is shown. **c**, BiFC analysis of PIF4–DELLA interaction. YFP, eYFP fluorescence; DAPI, 4,6-diamidino-2-phenylindole nuclei staining. **d**, Co-immunoprecipitation of the *Arabidopsis* GFP–RGA and PIF4 proteins. Plant extracts were immunoprecipitated with an anti-GFP antibody and detected by western blot using an antibody raised against PIF4 and the anti-GFP antibody.

The observation that the *phyB* hypocotyl phenotype is epistatic to *pif4* led to the proposal that PIF4 acts as a negative regulator of phyB signalling[3]. However, consistent with recent reports showing an additive function of PIF4 and PIF5 in hypocotyl elongation[18–20], we found that an additional mutation of the *PIF5* gene suppresses the

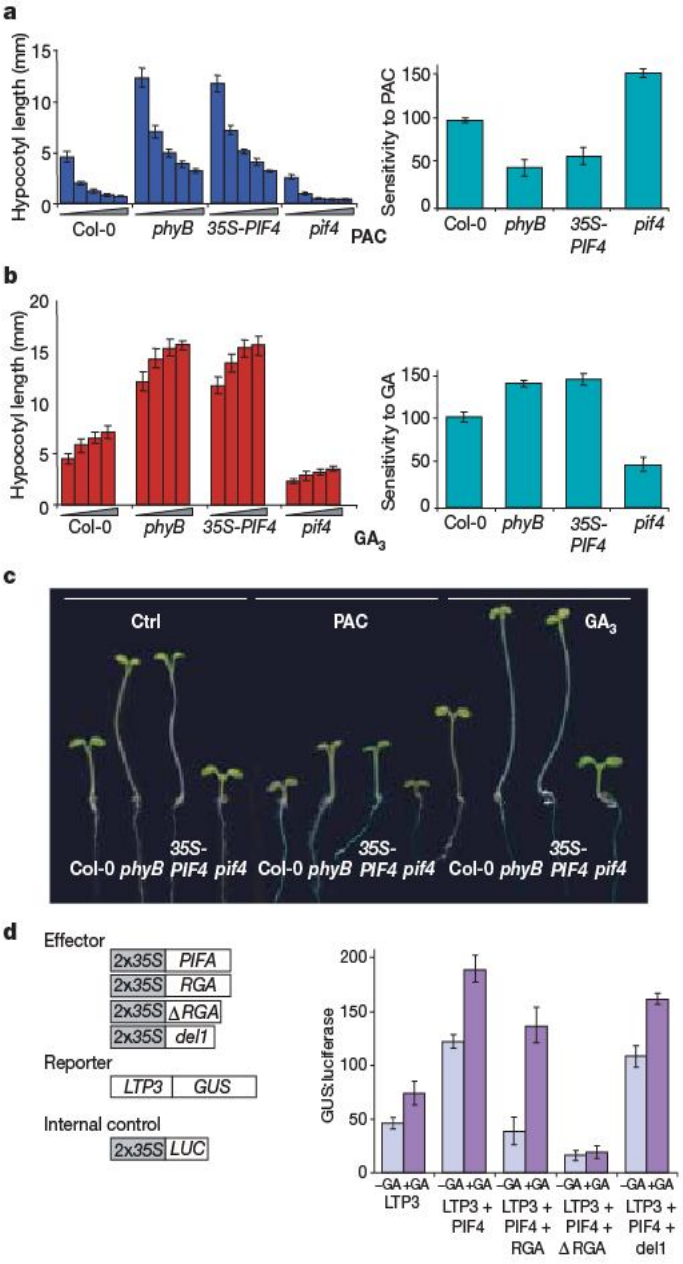

Figure 2 | Altered response to GA and paclobutrazol (PAC) treatments of *35S-PIF4* and *pif4* lines, and transient expression assays providing evidence for a block of PIF4 transcriptional activity by DELLAs. **a**, Hypocotyl lengths of Col-0, *phyB*, *35S-PIF4* and *pif4* seedlings grown in the presence of increasing concentrations (0, 0.025, 0.05, 0.1 and 0.2 μM) of the GA biosynthesis paclobutrazol (PAC) inhibitor. Right panel, relative response to PAC treatment. Means ± s.d. were obtained from 20 independent plants. Values are the mean of three independent experiments; error bars, s.d. between experiments. **b**, Hypocotyl lengths of seedlings grown under increasing concentrations of GA_3 (0, 2.5, 5.0 and 10 μM). Right panel, relative response to GA treatment. Means ± s.d. were obtained from 20 independent plants; error bars are as before. **c**, Phenotypes of the PAC- and GA-treated seedlings. **d**, Transient assays of PIF4 transcriptional activity in *Arabidopsis* cells. Constructs used in the experiment are shown on the left. Cells were bombarded with the *LTP3-GUS* reporter alone or co-bombarded with combinations of these constructs, as indicated. A *35S-LUC* (luciferase) construct was used as the internal control for transformation. Cells were incubated without (−GA) or with 50 μM (+GA) GA_3 and transcriptional activity measured as the ratio of GUS:LUC activity. Histogram columns represent the mean of eight biological replicates; error bars, s.e.m.

elongated phenotype of *phyB pif4* seedlings (Supplementary Fig. 4a, b). Hypocotyl growth of these lines correlates with levels of expression of transcripts *LTP3* (At5g59320) and β-expansin (At2g20750), found to be upregulated in *phyB* and *35S-PIF4* seedlings and repressed in the *pif4* mutant (Supplementary Fig. 4c). Further reduction of these transcripts in the *pif4 pif5* mutant confirmed an additive function of these factors. Interestingly, *phyB pif4 pif5* mutants remained taller than *pif4 pif5* seedlings, indicating that phyB-regulated factors other than PIF4 and PIF5 might also participate in hypocotyl growth control (see ref. 21).

A positive regulatory function of PIF4 was further supported by chromatin immuno-precipitation (ChIP) assays using lines expressing a fusion of PIF4 to the haemagglutinin (HA) antigen (PIF4–HA). PCR amplification of the upstream regions of genes differentially expressed in the *35S-PIF4* or *pif4 pif5* mutant lines showed that PIF4–HA bound exclusively the upstream region of upregulated genes with a G-box element in their promoters (Fig. 3a). Induced genes lacking a G-box or those corresponding to repressed genes were not amplified in these assays, demonstrating that these genes may be secondary targets of PIF4 activity. Interaction of PIF4 with its targets was strongly reduced in seedlings accumulating the DELLA repressors (PAC treated), whereas it was enhanced in seedlings treated with GAs to destabilize the DELLAs (Fig. 3a). Hence, these results provided experimental evidence for a role of DELLAs in blocking PIF4 DNA-binding ability *in vivo*. Additional evidence for such a sequestration mechanism was obtained by electrophoretic mobility-shift assay (EMSA) experiments using protein extracts of *Nicotiana benthamiana* leaves agroinfiltrated with the PIF4–HA or GFP–RGA constructs, or with a 1:1 mix of these *Agrobacterium* strains. Indeed, PIF4–HA bound an *LTP3* promoter fragment containing a G-box but co-expression of GFP–RGA abolished such binding activity (Fig. 3b) although it did not affect PIF4–HA levels (see Fig. 3b). Competition experiments with the cold probes showed that this binding activity requires an intact G-box.

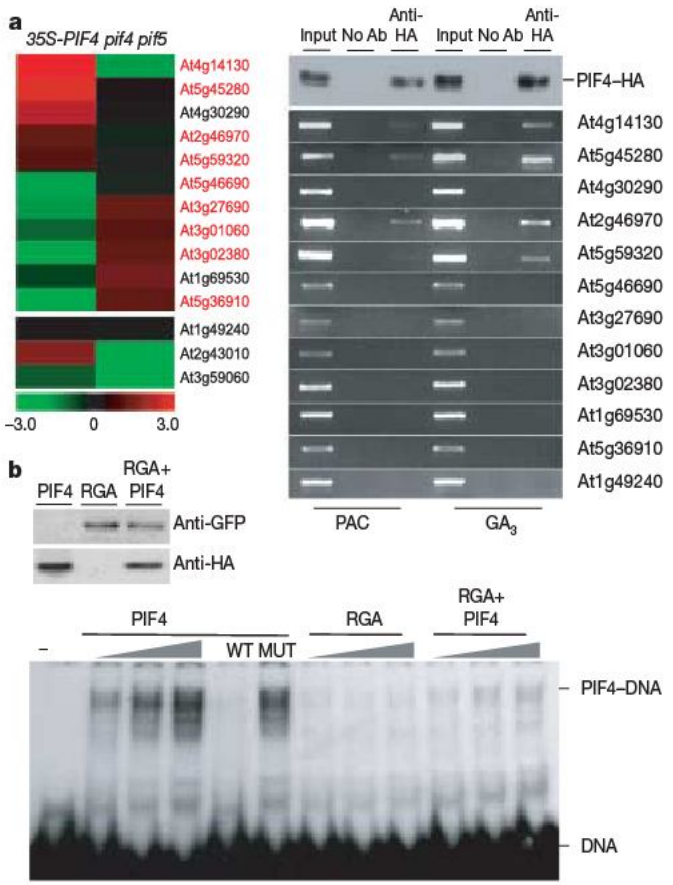

Figure 3 | Positive regulatory function of PIF4 and block of PIF4 DNA-binding ability by the DELLAs. **a**, Chromatin immunoprecipitation and promoter PCR amplification analysis, using PIF4–HA seedlings. Left panel, levels of expression of the selected genes in *35S-PIF4* and *pif4 pif5* lines. Colour scale represents fold-change (log). Genes exhibiting a G-box in their promoters are written in red. Right panel, PCR amplification of the immunoprecipitated PIF4–DNA complexes. Anti-HA indicates immunoprecipitation with an anti-HA antibody. Samples processed equally but without antibody (no Ab) were used as negative control. Seedlings were incubated overnight with 0.1 μM PAC or 25 μM GA_3 (GA) in the dark. Western detection was used to assess that similar amounts of the PIF4–HA protein (PIF4–HA) were recovered in both treatments. **b**, EMSA studies using an *LTP3* promoter fragment with a G-box element. *N. benthamiana* leaves infiltrated with the PIF4–HA and RGA–GFP constructs, or a 1:1 mixture of these *Agrobacterium* strains, were used to obtain the proteins. The abundance of the PIF4 and RGA proteins in these extracts was evaluated by western blot with anti-HA (PIF4) and anti-GFP (RGA) antibodies. The *LTP3* DNA probe was incubated with increasing amounts of the proteins as indicated. −, incubation without protein extract. WT and MUT, competition with a 100-fold excess of cold wild-type (WT) and mutated (MUT) probes.

Overall, our results are consistent with a positive-control function of PIF4 on hypocotyl growth and indicate that phyB signalling might repress hypocotyl growth by inhibiting PIF4 transcriptional activity. Nuclear translocation of phytochromes was in fact reported to induce proteasome-mediated degradation of the PIF3 and PIF1/PIL5 factors[22,23]. Therefore it is possible that phyB exerts a similar control on PIF4. Our experiments indeed established that PIF4–GFP nuclear fluorescence was high in etiolated seedlings but rapidly disappeared on light irradiation (Fig. 4a). Seedling pre-treatment with the 26S proteasome inhibitor MG132 stabilized the protein in the light and, more importantly, light-dependent destabilization of the protein was not observed in *phyB* mutant seedlings, showing that phyB signalling is required for proteasome-mediated degradation of PIF4 (Fig. 4). Similar results have been reported recently for PIF5, corroborating our findings[24].

The functional significance of these results was further investigated by overexpressing the PIF4 factor in a 20-oxidase GA-deficient background (*20ox*) or in seedlings expressing the *gai* dominant allele (*35S-gai*), which lacks the DELLA domain and confers a GA-insensitive phenotype[13]. PIF4 overexpression in the *20ox* or *gai* mutants resulted in notable hypocotyl growth compared to the mutations alone (Fig. 4b), demonstrating that this transcription factor is able to rescue partly the growth restraint imposed by DELLA accumulation. GA treatment, in addition, fully restored growth of *35S-PIF4 20ox* hypocotyls by inducing DELLA destabilization, but it did not alter growth of *35S-PIF4 gai* seedlings, which accumulate a stable form of these repressors (Fig. 4b).

Collectively, our data are indicative of a positive function of the PIF4 and PIF5 factors in activated expression of cell elongation genes. In the light, phyB negatively regulates PIF4 transcriptional activity, by targeting degradation of this transcription factor by the 26S proteasome pathway (Fig. 4c). DELLAs repress transcriptional activity of the PIF factors by interacting with the bHLH DNA-recognition domain and sequestering these factors into an inactive complex, unable to bind DNA (Fig. 4c). Consistent with this mode of action, stabilization of the DELLA proteins represses PIF4-mediated cell growth, whereas GAs induce elongation growth by destabilizing these repressors, allowing accumulation of free PIF4 in the nucleus and the activation of PIF4-regulated genes (Fig. 4c). Inactivation of this PIF factor by DELLA protein interaction or by phyB-mediated destabilization actually explains the intermediate hypocotyl lengths of double *phyB ga1.3*, *phyB ga4*, or *phyB gai* mutants[25] or those of the transgenic *35S-PIF4 20ox* and *35S-PIF4 gai* lines (Fig. 4b), which were not previously understood in the context of a simple genetic pathway.

PIFs are members of a subfamily of bHLH proteins with highly related DNA-binding domains and it is therefore possible that DELLAs block transcriptional activity of all members of this gene family. DELLA repressor interaction with PIF3 is in fact described in a companion report[21]. Notably, whereas PIF3 and PIF4 primary function is in hypocotyl elongation control[3,26], other PIFs have been

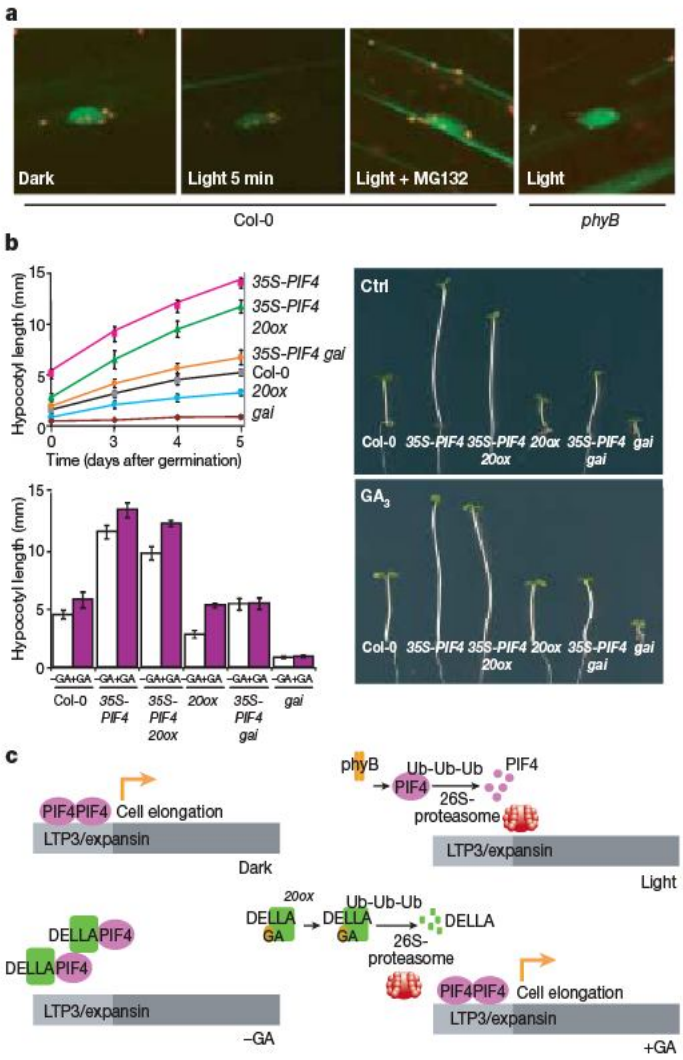

Figure 4 | PHYB-mediated degradation of PIF4 and intermediate hypocotyl lengths of *35S-PIF4 20ox* and *35S-PIF4 gai* seedlings. **a**, Confocal fluorescence of nuclei of Col-0 and *phyB* transgenic lines expressing the PIF4–GFP construct. Seedlings were incubated overnight in the dark and GFP fluorescence visualized either directly (dark) or after 5 min of irradiation with white light (light 5 min). Light induced a decline in GFP fluorescence in Col-0 lines but not in the *phyB* mutant (*phyB*). Treatment with the MG132 proteasome inhibitor stabilized GFP fluorescence of Col-0 seedlings in the light (light + MG132). **b**, Hypocotyl lengths of the double *35S-PIF4 20ox* and *35S-PIF4 gai* lines. Accumulation of DELLAs in these lines leads to intermediate hypocotyl phenotypes. Treatment with GA_3 rescued hypocotyl growth in the *PIF4-OE 20ox* lines but did not affect growth of *35S-PIF4 gai* seedlings, accumulating a stable DELLA. Hypocotyl lengths at different days of germination (upper left panel) and growth response induced by 2.5 μM GA_3 (lower left panel; phenotypes in right panels). Values represent the mean of 10 plants; error bars, s.d. **c**, Model for direct PIF4 integration of both light and GA signals. In the light, phyB induces destabilization of PIF4. DELLAs interact with PIF4 and repress its DNA-binding ability. GAs trigger proteasome degradation of the DELLA repressors and allow accumulation of free PIF4, promoting PIF4-activated gene expression.

reported to control other light-regulated responses such as chlorophyll biosynthesis[27] or seed germination[28]—processes that are also known to be modulated by DELLAs. Hence, competitive interaction with members of the PIF family of transcription factors might be a prevailing mechanism for DELLA function, serving to explain the great diversity of responses controlled by these repressors.

METHODS SUMMARY

Plant mutants and transgenic lines. Descriptions of mutants and transgenic lines used in this work is given in Methods. Double and triple mutations were genotyped using the primers indicated in Supplementary Table 1. The *LTP3* promoter and the *RGA*, *GAI*, *RGL1* and *RGL3* genes were amplified from Col-0 genomic DNA using the primer combinations listed in Supplementary Table 3. *PIF4* was amplified from leaf RNA. Constructs and fusions to the GFP and enhanced YFP fluorescent proteins are described in Methods.

Protein interaction assays. The yeast GAL4 system was employed for two-hybrid screening with DELLAs. For pull-down assays, a GST–RGA fusion bound to glutathione-*Sepharose* beads was incubated with ^{35}S-labelled phyA, PIF3 and PIF4 proteins. BiFC assays were performed as described in Methods. For co-immunoprecipitation experiments, extracts of GFP–RGA seedlings were incubated with an anti-GFP antibody (Santa Cruz), immunoprecipitated with protein G agarose (Sigma) and analysed by western blot using an antibody raised against the PIF4 protein.

ChIP, transactivation and gel-shift assays. Chromatin immunoprecipitation (ChIP) was performed as described[29]. PIF4–HA seedlings and an anti-HA antibody (Santa Cruz) were used in these assays. Transient expression and gel-shift assays were performed as described[30]. Details for constructs and bombardment conditions are given in the Methods. A fragment of the *LTP3* promoter (At5g59320) with a G-box was used as a DNA probe for retardation. Leaves agro-infiltrated with the *PIF4–HA* and *GFP–RGA* constructs were used to obtain the proteins.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions M.d.L. performed the bimolecular complementation assays, EMSA and co-immunoprecipitation studies and analysed phyB-mediated destabilization of the PIF4 protein; he was also co-responsible for the transient expression experiments, together with E.T. J.-M.D. performed the expression analyses and the selection of double and triple mutant lines and the ChIP experiments, in collaboration with J.M.I.-P. M.R.-F. performed the two-hybrid screening experiments and pull-down assays and together with M.P. mapped the interacting domains in both proteins. S.L. and C.F. provided the PIF4–HA lines and an antibody raised against the PIF4 protein. M.A.B. provided the *35S-gai* lines and helped with critical discussions on the work. S.P. designed experiments, supervised the work and wrote the manuscript.

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LETTERS

Distinct roles of the FliI ATPase and proton motive force in bacterial flagellar protein export

Tohru Minamino[1,2] & Keiichi Namba[1,2]

Translocation of many soluble proteins across cell membranes occurs in an ATPase-driven manner. For construction of the bacterial flagellum responsible for motility, most of the components are exported by the flagellar protein export apparatus[1,2]. The FliI ATPase is required for this export[3], and its ATPase activity is regulated by FliH[4]; however, it is unclear how the chemical energy derived from ATP hydrolysis is used for the export process. Here we report that flagellar proteins of *Salmonella enterica* serovar Typhimurium are exported even in the absence of FliI. A *fliH fliI* double null mutant was weakly motile. Certain mutations in FlhA or FlhB, which form the core of the export gate, substantially improved protein export and motility of the double null mutant. Furthermore, proton motive force was essential for the export process. These results suggest that the FliH–FliI complex facilitates only the initial entry of export substrates into the gate, with the energy of ATP hydrolysis being used to disassemble and release the FliH–FliI complex from the protein about to be exported. The rest of the successive unfolding/translocation process of the substrates is driven by proton motive force.

The flagellar export apparatus consists of six integral membrane proteins (FlhA, FlhB, FliO, FliP, FliQ and FliR) and three soluble proteins (FliH, FliI and FliJ), and is thought to be located at the base of the flagellum[1,2]. Export of bacterial flagellar proteins has characteristics in common with the type III secretion system (T3SS) of virulence factors of pathogenic bacteria, and these two systems actually consist of many homologous component proteins[5].

The soluble export component FliI is an ATPase[3]. Because mutants lacking the ATPase activity cannot export flagellar proteins, FliI was thought to convert chemical energy released by ATP hydrolysis into mechanical work necessary for the export[3]. FliI shows extensive similarity to the α/β subunits of proton-driven F_0F_1-ATPase for its entire molecular structure[6], although sequence similarity is limited to their respective ATPase domains[7,8]. Unlike F_1-ATPase, however, which forms the $\alpha_3\beta_3$ hexameric ring, FliI self-assembles into a homohexamer[9,10]. When FliI oligomerization is suppressed by a small deletion in its amino-terminal region, flagellar protein export does not occur efficiently, suggesting that FliI hexamerizes on docking to the export gate made of the six integral membrane components, for which the cytoplasmic domains of FlhA and FlhB are thought to form the docking platform[11]. FliI binds to FlgN (chaperone) and to a FlgN–FlgK (HAP1) complex[12], suggesting that FliI has a critical role in substrate recognition as well. As *Salmonella* InvC—a virulence T3SS homologue of FliI—binds to chaperone–effector complexes and induces chaperone release from, and unfolding of, the effector to be secreted in an ATPase-dependent manner[13], FliI has been thought to act in a similar manner.

FliH binds to the extreme N-terminal region of FliI to form the $FliH_2$–FliI complex in the cytoplasm, and suppresses FliI oligomerization and ATPase activity[4,8]. However, as FliI cannot efficiently dock to the export gate when FliH is missing[14], FliH is postulated to provide a link between ATP hydrolysis by FliI and flagellar protein export, although it is not known how this is done. We therefore investigated this by characterizing a *Salmonella fliH fliI* double null mutant and by isolating gain-of-function mutants from this mutant.

A *fliH* null mutant was weakly motile whereas a *fliI* null mutant was non-motile[14,15]. Notably, when both FliH and FliI were missing (Fig. 1b, lane 4), the cells formed swarms on soft agar plates after prolonged incubation (Fig. 1a). Consistently, intact flagella as well as immature flagellar structures were occasionally observed by electron microscopy (data not shown). We then examined the effect of FlhB deletion, which makes wild-type cells non-motile (Fig. 1a). The motility of the double null mutant was totally abolished by a *flhB*::Tn*10* mutation (Fig. 1c), indicating that some flagella are produced at a low probability even in the absence of FliH and FliI, but in a manner that still requires FlhB.

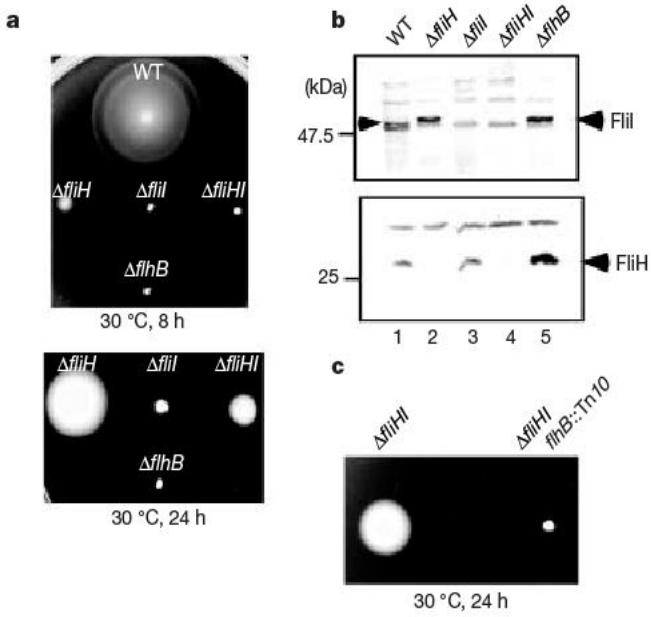

Figure 1 | Characterization of a *fliH fliI* double null mutant. a, Swarming motility of SJW1103 strain (wild type, WT), and MKM11 (Δ*fliH*), MKM30 (Δ*fliI*), MMHI001 (Δ*fliH fliI*, indicated as Δ*fliHI*) and MKM50 (Δ*flhB*) mutant strains on soft agar plates. **b**, Immunoblotting, using anti-FliH and anti-FliI antibodies, of whole cell proteins. Weak bands just below those of FliI are due to nonspecific reactions. A slight downward shift of the FliI band in lane 1 is caused by an invisible band of a large amount of FliC (51.5 kDa) just above it. **c**, Effect of a *flhB*::Tn*10* mutation on motility of the Δ*fliH fliI* mutant.

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FliH and FliI interact not only with FlhA and FlhB but also with flagellar chaperones such as FliJ and export substrates, leading to the proposal that the FliH–FliI complex is responsible for delivery of the substrates to the export gate[4,11,12,14]. To test this, gain-of-function mutants were isolated from the *fliH fliI* double null mutant by streaking an overnight culture out on soft agar plates, incubating at 30 °C for 2 days and looking for motility haloes emerging from the streak. In total 22 pseudo-revertants were obtained. When we analysed the secretion of FliC (flagellin) into the culture supernatant by Coomassie brilliant blue staining, FliC was seen on SDS–polyacrylamide gel electrophoresis gels from the MMHI0117 strain, but not from the others (data not shown). Therefore, we decided to carry out further characterization of MMHI0117. The motility of MMHI0117 was significantly better than that of the parent mutant although not as good as that of wild type (Fig. 2a). Most cells of this strain had a couple of long flagellar filaments, whereas wild-type cells produce five or more (data not shown). Most flagellar proteins were detected in the culture supernatant of this mutant (Fig. 2b, lane 6). The amounts of FlgD (hook cap protein) and FliK (hook-length control protein) secreted by this pseudo-revertant were even larger than those of the wild type, whereas the secretion levels of FlgG (distal rod protein) and FlgE (hook protein) were threefold and tenfold lower than wild-type levels, respectively. Consistently, flagellar basal bodies, often with the hook and filament attached, were observed by electron microscopy (Fig. 2c). The secretion levels of these proteins were restored to wild-type levels by introduction of a plasmid encoding FliH and FliI into the mutant (Supplementary Fig. 1). These results indicate that the export of FlgE and FlgG depends on the FliH–FliI complex more strongly than the export of FlgD and FliK. As all of these proteins belong to the same rod-type and hook-type export class[16], the FliH–FliI complex not only guides these proteins to the export gate but also controls their export order and levels. The cellular levels of FliC, FlgK and FlgL (HAP3) in the MMHI0117 strain were much lower than those in the wild type because expression of these genes occurs only after hook completion[17], which is evidently retarded in this strain (Fig. 2b).

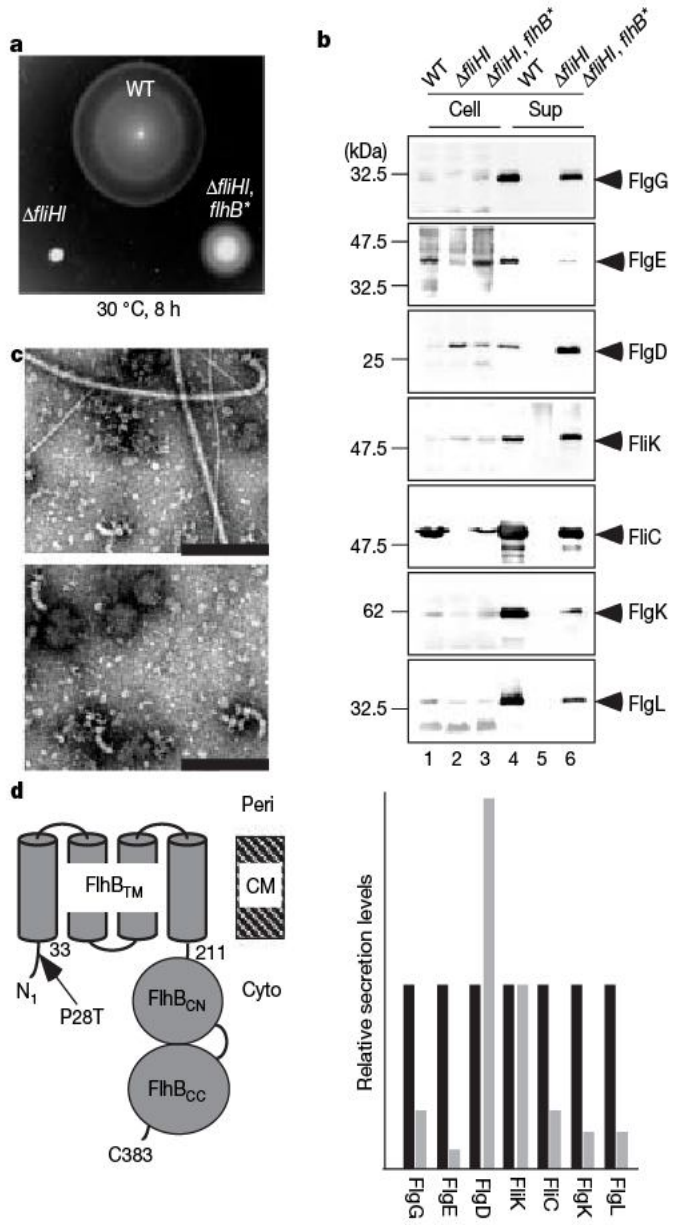

Figure 2 | Isolation of pseudo-revertants from the Δ*fliH fliI* mutant. **a**, Motility of wild type, Δ*fliH fliI* mutant (Δ*fliHI*) and its pseudo-revertant (Δ*fliH fliI, flhB*(P28T), indicated as Δ*fliHI, flhB**). **b**, Secretion analysis of FlgG, FlgE, FlgD, FliK, FliC, FlgK and FlgL by immunoblotting. 'Cell' and 'Sup' indicate whole cell proteins and culture supernatant fractions, respectively. Relative secretion levels are normalized to the wild-type level of each protein. Black bar, wild type; grey bar, pseudo-revertant. **c**, Electron micrographs of the flagella and basal bodies isolated from Δ*fliH fliI, flhB*(P28T). Scale bar, 200 nm. **d**, Position of a suppressor mutation in FlhB. CM, cytoplasmic membrane; cyto, cytoplasm; peri, periplasmic space.

P22-mediated genetic mapping[18] showed that the gain-of-function mutation lies in the *flhBAE* operon (data not shown). DNA sequencing identified a missense mutation, P28T, near the N terminus of FlhB, close to the interface with the first membrane span (Fig. 2d). The position of this P28T mutation was very close to the positions of the FliH-bypass *flhB* mutations isolated previously[14]. Therefore, we tested whether the FliH-bypass *flhB* and *flhA* mutations can improve motility of the *fliH fliI* double mutant. These mutations, as represented here by a *flhA*(V404M) mutation, considerably enhanced both motility and flagellar protein export (Supplementary Fig. 2), although their suppression abilities were much weaker than that of the *flhB*(P28T) mutation.

Salmonella T3SS virulence factors are secreted by the flagellar export apparatus in the absence of their chaperones[19]. As InvC and SsaN are the T3SS homologues of FliI in *Salmonella*[20,21], there is a possibility that InvC and/or SsaN may complement the *fliH fliI* double null mutants for flagellar protein export, although SsaN is not expressed under our experimental condition[21]. However, this was ruled out by our observations that neither motility nor protein export of the pseudo-revertant was abolished by InvC or SsaN deletions (Supplementary Fig. 3). Therefore, we conclude that the gain-of-function mutations in FlhA and FlhB increase the probability of entry of flagellar proteins into the export gate, thereby increasing export efficiency. An increased gate-opening probability for higher efficiency of protein entry could be deleterious to the cells owing to leakage of small solutes. However, the growth of the mutants was normal (Supplementary Fig. 4a), suggesting that the export gate is not always open. It is likely that the interaction of export substrates with the gate, with or without the FliH–FliI complex, induces opening of the pore.

Unlike the *fliH* null and the *fliH fliI* double null mutants, the *fliI* null mutant was non-motile (Fig. 1a), indicating that FliH inhibits flagellar protein export in the absence of FliI. To test whether the *flhB*(P28T) mutation would also suppress the *fliI* null mutation, we analysed the motility of the pseudo-revertant transformed with a plasmid encoding FliH (Supplementary Fig. 5). The motility gained by the *flhB*(P28T) mutation was significantly suppressed, suggesting that the docking of free FliH to the FlhA–FlhB platform interferes with the entry of export substrates into the export gate, even in the presence of the *flhB*(P28T) mutation.

As it has been shown that *Yersinia enterocolitica* type III secretion is inhibited considerably by treatment with the protonophore carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)[22], and that flagellar formation is also dependent on proton motive force (PMF)[23], we investigated whether flagellar protein export is still dependent on PMF in the gain-of-function mutant as well as wild-type cells. The rate of cell growth decreased when CCCP concentration increased,

and 25 μM CCCP immediately caused growth arrest (Supplementary Fig. 4b). As expected, the flagellar motor rotation, which is driven by the PMF[1], was abolished by 25 μM CCCP treatment, indicating that the PMF is significantly collapsed (data not shown). The intracellular levels of FlgD were maintained even at 25 μM (Fig. 3); however, the levels of FlgD secretion by both wild-type and mutant cells decreased significantly above 10 μM CCCP and diminished at 25 μM (Fig. 3). In agreement with previous data[22], the intracellular ATP level did not change on CCCP treatment within 1 h (Supplementary Fig. 4c). These results indicate that PMF is absolutely essential for FlgD export regardless of the presence or absence of FliH and FliI.

The MotA–MotB complex acts as a proton conductive pathway to couple proton influx with flagellar motor rotation[1]. Mutations in MotA or MotB do not affect flagellar formation while the motor is paralized[1]. Consistently, deletions of these two proteins do not interfere with flagellar protein export even in the gain-of-function mutant (data not shown).

Both ATP and PMF seem to be required for pre-protein translocation in *Escherichia coli*, where ATP is essential for the initial step of protein translocation and PMF increases the rate of translocation[24]. SecA is the ATPase that uses the energy of ATP binding and hydrolysis to drive pre-protein translocation into and across the cytoplasmic membrane[24]. Each catalytic cycle of SecA permits the stepwise translocation of pre-proteins across the membrane[25,26]. In contrast, we have shown that ATP hydrolysis by FliI is not absolutely required for flagellar protein export. As the diameter of the central channel of the flagellum—which is the export path for flagellar proteins—is only 2 nm[27], proteins to be exported must be largely unfolded for entry into and translocation through the channel. Our observations—in particular, levels of FlgD and FliK secretion by a gain-of-function mutant that exceed wild-type levels (Fig. 2c)—suggest that the successive process of unfolding and translocation of export substrate is driven by PMF. The role of the FliH–FliI complex for efficient export would probably be to increase the initial docking efficiency of the N-terminal segments of export substrates into the export gate through specific interactions between the $FliH_x$–$FliI_6$ complex and the FlhA–FlhB platform. As the binding of the $FliH_x$–$FliI_6$ complex without ATPase activity inhibits the export process[4], the energy of ATP hydrolysis seems to be used to facilitate the release and disassembly of the $FliH_x$–$FliI_6$ complex from the export gate and the protein about to be exported, in order for the subsequent PMF-driven, successive process of unfolding of the protein and translocation of the polypeptide chain through the gate by the export gate complex to proceed without retardation, as well as for efficient use of the $FliH_2$–FliI complexes for the next cycle of initial docking (Fig. 4). Because N-terminal segments of export substrates containing export signals are all natively unfolded in the monomeric form of the proteins[28], the energy of ATP hydrolysis would not be required for the process of initial docking and entry to the gate.

Considering the many similarities between the flagellar export apparatus and F_0F_1-ATPase, such as almost identical structures of

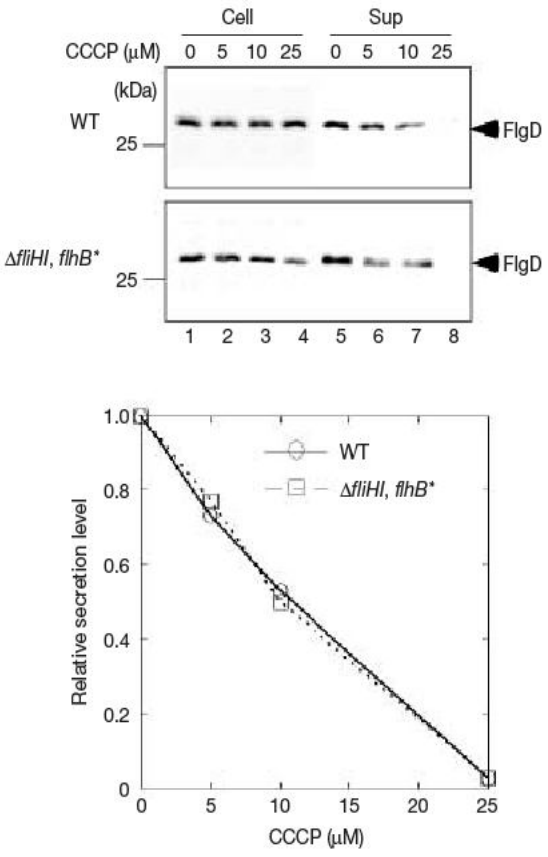

Figure 3 | Effect of CCCP on FlgD secretion. **a**, Immunoblotting, using the anti-FlgD antibody, of whole cell proteins and culture supernatant fractions prepared from wild-type (WT) and a gain-of-function mutant (Δ*fliH fliI*, *flhB**) grown at 30 °C in the presence of 0, 5, 10 and 25 μM CCCP. **b**, The decay in the secretion levels shown by plotting band densities of the two blots, which are normalized for the cellular FlgD levels. These data are the average of three independent experiments. The experimental errors are within 10%.

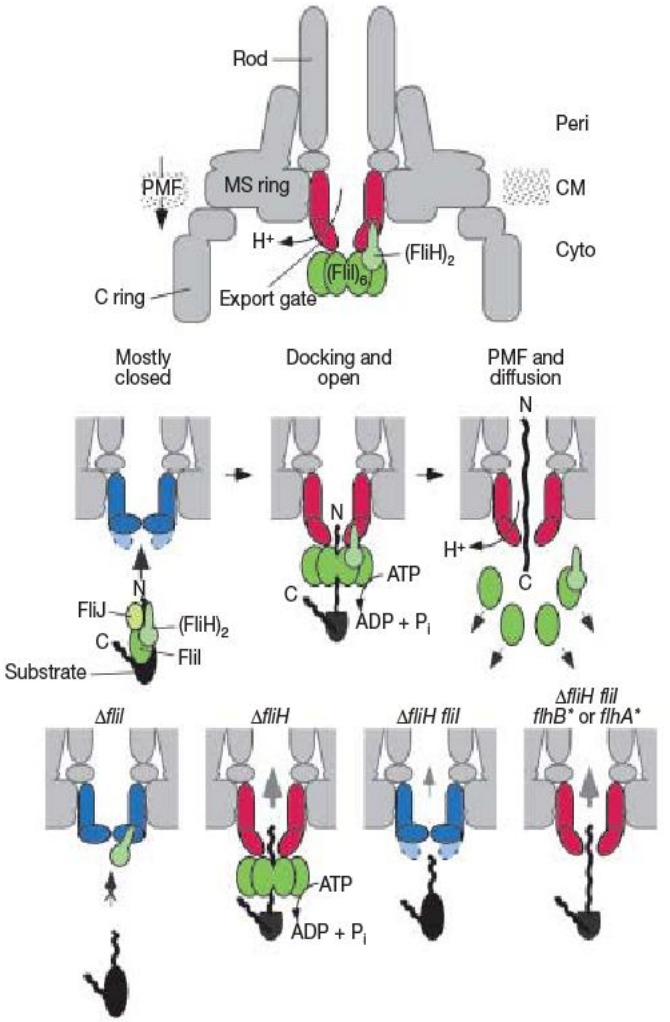

Figure 4 | Model for flagellar protein export. Top panel: The flagellar protein export apparatus in the basal body. Middle panel: in wild type, the $FliH_2$–FliI complex delivers export substrates to the export gate along with FliJ (left). Once the N-terminal segment of a substrate is docked by formation of the $FliH_x$–$FliI_6$ complex (middle), ATP hydrolysis induces dissociation of the $FliH_x$–$FliI_6$ complex and successive unfolding and translocation of the substrates is driven by the PMF (right). Bottom panel: protein translocation is inhibited by FliH in the *fliI* mutant (left). The *fliH* mutant retains the export function to some extent (middle left). The *fliH fliI* double mutant also retains the export function, albeit at a low level (middle right). Gain-of-function mutations increase the efficiency of substrate entry (right). P_i, inorganic phosphate.

FliI and the α/β subunits[6], the PMF-driven functions of the export gate and the F_0 portion, and sequence/functional similarities between FliH and the δ/b subunits[29], these two remotely related systems may be similar to each other for their entire structural architectures.

METHODS SUMMARY

Salmonella strains and plasmids used in this study are listed in Supplementary Table 1. L-broth (LB) and soft tryptone agar plates were prepared as described[30]. Ampicillin and tetracycline were added as needed at a final concentration of 100 μg ml^{-1} and 15 μg ml^{-1}, respectively. Motility assays were carried out on soft agar plates as described before[4]. Export assays were done as described previously[30].

The hook–basal bodies were purified and negatively stained with 3% phosphotungstic acid (pH 4.5) on carbon-coated copper grids. Micrographs were recorded at a magnification of ×25,000 with a JEM-1011 transmission electron microscope (JEOL) operated at 100 kV.

The cellular levels of ATP were measured using the ATP bioluminescence assay kit CLS II as described previously[22].

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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LETTERS

Energy source of flagellar type III secretion

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Bacterial flagella contain a specialized secretion apparatus that functions to deliver the protein subunits that form the filament and other structures to outside the membrane[1]. This apparatus is related to the injectisome used by many gram-negative pathogens and symbionts to transfer effector proteins into host cells; in both systems this export mechanism is termed 'type III' secretion[2,3]. The flagellar secretion apparatus comprises a membrane-embedded complex of about five proteins, and soluble factors, which include export-dedicated chaperones and an ATPase, FliI, that was thought to provide the energy for export[1,4]. Here we show that flagellar secretion in *Salmonella enterica* requires the proton motive force (PMF) and does not require ATP hydrolysis by FliI. The export of several flagellar export substrates was prevented by treatment with the protonophore CCCP, with no accompanying decrease in cellular ATP levels. Weak swarming motility and rare flagella were observed in a mutant deleted for FliI and for the non-flagellar type-III secretion ATPases InvJ and SsaN. These findings show that the flagellar secretion apparatus functions as a proton-driven protein exporter and that ATP hydrolysis is not essential for type III secretion.

Flagellar assembly begins with structures in the cytoplasmic membrane and proceeds through steps that add the exterior structures in a proximal-to-distal sequence (Fig. 1)[1]. Assembly of the rod, hook and filament requires the action of the secretion apparatus, which transports the needed subunits into a central channel through the structure that conducts them to their site of incorporation at the tip (Fig. 1). Flagellar export is notably fast: in the early stages of filament growth flagellin is delivered at a rate of several 55 kDa subunits per second[5].

ATP hydrolysis by FliI was thought to provide the energy for export because mutations that delete or reduce the activity of FliI block flagellar synthesis at the stage of rod assembly[1,4,6] (Fig. 1). Homologues of FliI also occur in the type III secretion apparatus of injectisomes and are usually assumed to energize export in those systems as well. Some evidence for a different view has also been reported: it was observed that type III secretion in *Yersinia enterocolitica* was prevented by the protonophore CCCP[7], and it was shown that the secretion ATPase InvC of *Salmonella* functions to dissociate export substrate from the chaperone[8], a role distinct from transport itself. The energy source for type III secretion thus remains uncertain.

To address the energy requirements for type III secretion, we first measured the effect of the uncoupler CCCP on flagellar export in *S. enterica*, assayed by accumulation of the export substrate FlgM in the medium. FlgM export was prevented by 10 μM or more CCCP (Fig. 2a). Overall cellular energy levels seemed unaffected, because cells grew normally in 10 μM CCCP (growth data not shown) and ATP levels were unchanged (Supplementary Fig. 1). The effect was reversible: FlgM export was largely restored following a 30-min washout of the 10 μM CCCP (Fig. 2b). FlgM inhibits transcription of its own gene, and so reduced FlgM export might partially reflect decreased cellular levels of the protein[9]. To circumvent this autoinhibitory effect the experiment was repeated with a strain in which *flgM* was placed under control of a non-native (P_{araB}) promoter. In this strain, the cytoplasmic level of FlgM remained nearly constant, whereas FlgM secretion was again prevented by 10 μM or more CCCP (Fig. 2c).

The maintenance of normal ATP levels in the presence of CCCP was noted previously in experiments with *Y. enterocolitica*[7] and is thought to be due to a regulatory mechanism that inhibits the hydrolytic activity of the ATP synthase when the membrane is de-energized[10]. Because this protective mechanism may not act instantaneously, cellular ATP levels might undergo a transient drop following CCCP treatment that would escape detection in our measurements. To rule out such an effect, we measured FlgM export in a Δ*atpA* strain that lacks a major subunit of the ATP synthase. FlgM secretion was again prevented by 10 μM or higher CCCP, and ATP levels were unaffected (Fig. 2d, e).

The flagellum exports more than a dozen substrates, which are classified as early or late according to whether they are secreted during assembly of the hook/basal-body or the filament[1] (Fig. 1). To test the generality of the PMF requirement we examined the effect of CCCP on secretion of additional substrates, assayed by their accumulation in the culture medium[11]. We observed the early substrate FliK and the late-export substrates FlgK, FlgL and FliC in culture supernatants of LB-grown cells. Accumulation of all four substrates was prevented by treatment with 20 μM CCCP (Fig. 2f). The band between FliC and FlgK does not correspond to the size of a known flagellar protein and is likely to be a stable protein secreted in (relatively) low amounts.

CCCP functions as a proton carrier to discharge both the electric potential ($\Delta\psi$) and concentration (ΔpH) components of the PMF. To examine the contribution of the $\Delta\psi$ component separately, we measured FlgM secretion in cells treated with the K^+-ionophore valinomycin. In medium containing 150 mM KCl, FlgM secretion was inhibited by 10 μM valinomycin (Fig. 3a). Thus, the electrical potential component seems essential for export under the conditions of this experiment (extracellular pH = 7.3). Next, the ΔpH component of the gradient was discharged by the weak acid acetate (34 mM), which crosses the membrane in neutral (protonated) form and releases protons inside the cell. At an external pH of 7, FlgM secretion was not affected by treatment with acetate (Fig. 3b), indicating that flagellar export can be supported by $\Delta\psi$ alone. At an external pH of 5, acetate prevented secretion (Fig. 3b), presumably owing to acidification of the cytoplasm and the resulting protonation of one or more functionally important acidic groups. A similar effect was reported with the flagellar motor, which ceased rotating when the cytosolic pH was lowered to 5 (ref. 12).

We characterized export requirements further using an assay based on secretion of a FlgE–Bla (hook/β-lactamase) fusion protein. Cells were deleted for the rod proteins FlgB and FlgC (Fig. 1) to direct the

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fusion protein into the periplasm, allowing export to be quantified by the MIC (minimum inhibitory concentration) for ampicillin[13]. The MIC value was reduced by uncoupler, from a value of 25 μg ml^{-1} in the untreated control to about 4 μg ml^{-1} in 30 μM CCCP (Table 1, and Supplementary Information).

If energy for flagellar transport comes from the proton gradient, then ATP hydrolysis by FliI may be less important than has been supposed. To examine the FliI requirement more closely we measured FlgE–Bla export in a strain deleted for *fliI* and the flanking genes *fliH and fliJ*. FliH is a regulator of FliI[14] and FliJ interacts with the FliHI complex and with other export components[15,16]. The MIC measured for the Δ*fliHIJ* strain was 12 μg ml^{-1}, reproducibly larger than that of a negative-control strain lacking the MS-ring gene *fliF* (<3 μg ml^{-1}) or a strain with all the flagellar genes repressed by

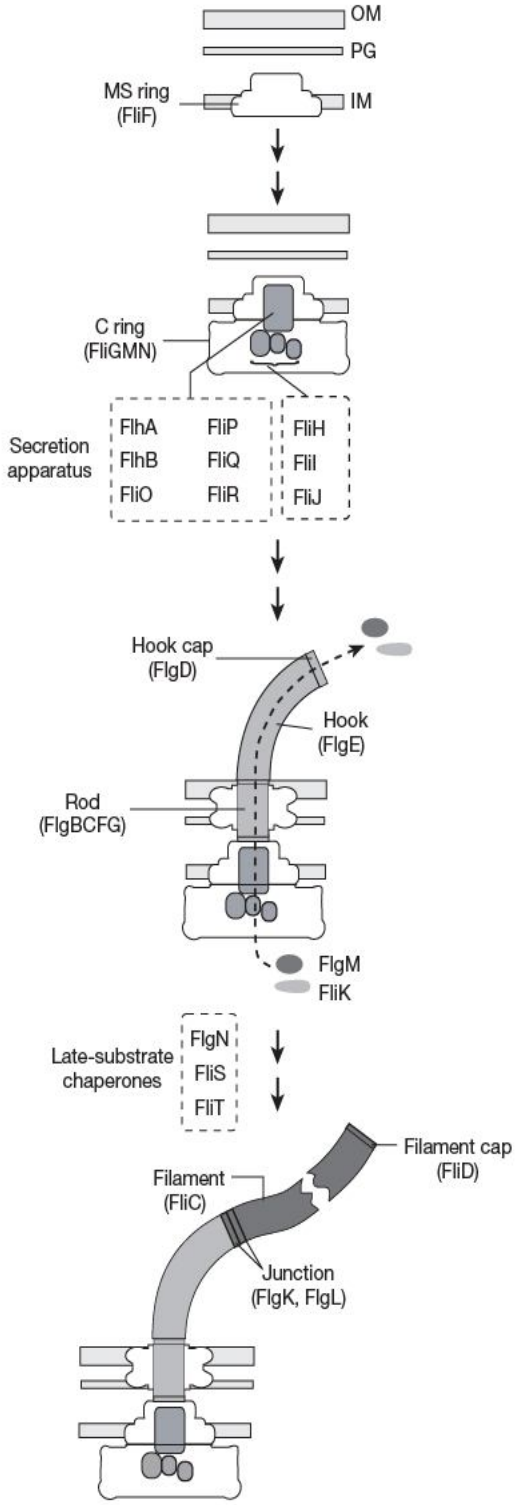

Figure 1 | Steps in flagellar assembly. Dashed boxes indicate the proteins that function in flagellar secretion, either in the membrane-bound part of the apparatus or in delivery of substrate. Flagellar components that depend on export are indicated in light- (early substrates) or dark- (late substrates) grey; these include the structural proteins that form the rod, hook and filament, the transcriptional regulator FlgM, and the hook-length regulator FliK. OM, outer membrane; PG, peptidoglycan; CM, cytoplasmic membrane.

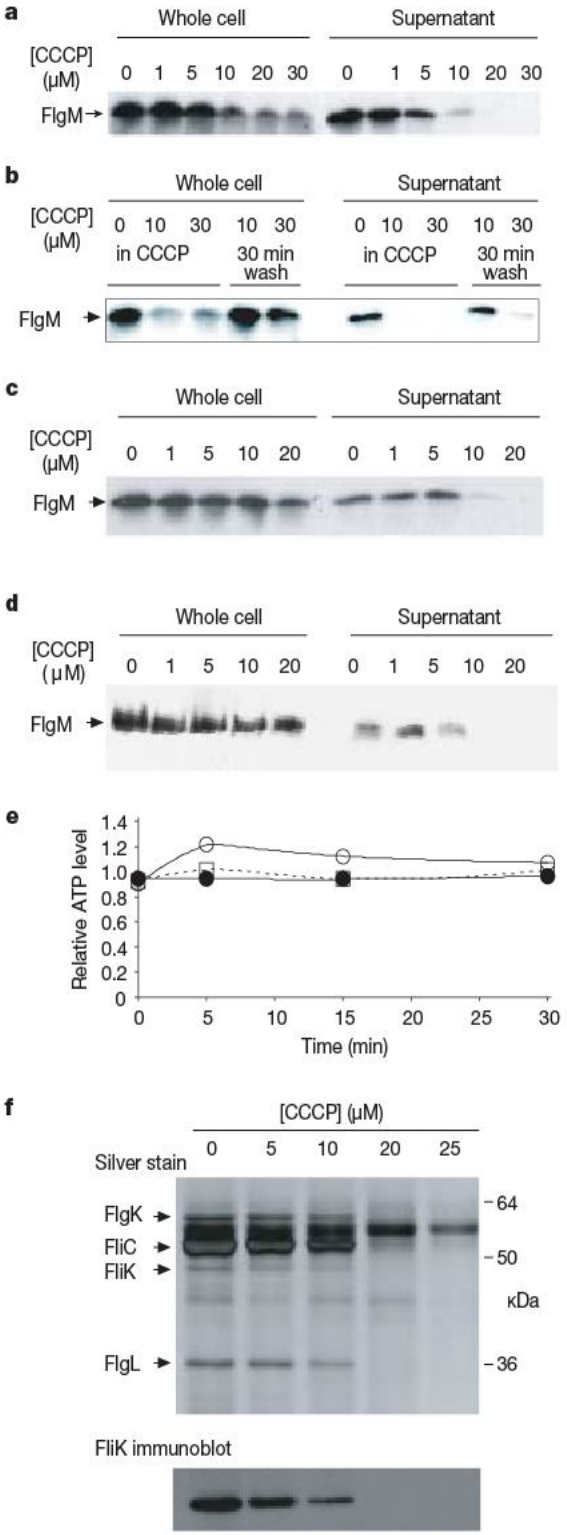

Figure 2 | Inhibition of FlgM secretion by CCCP. **a**, Secretion in *Salmonella* strain TH3730 (Tet-inducible *flhDC*). **b**, Partial restoration of export following a 30-min wash into CCCP-free buffer. **c**, Secretion in strain TH10874 (arabinose-inducible *flgM*). **d**, Inhibition of FlgM secretion by CCCP in an ATP-synthase defective (Δ*atpA*) strain (TH11802). **e**, ATP levels in the Δ*atpA* mutant at various times following treatment with CCCP. Open circles, no treatment; open square, 10 μM CCCP; and filled circles, 30 μM CCCP. **f**, Inhibition of secretion of other flagellar substrates (FlgK, FlgL, FliC and FliK) by CCCP.

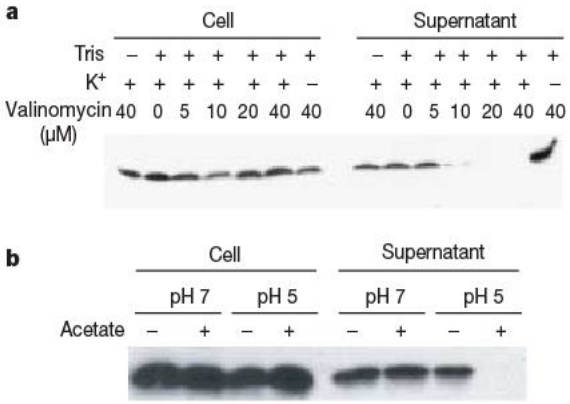

Figure 3 | Effect of Δψ and ΔpH on FlgM export. **a**, Inhibition of FlgM secretion by valinomycin and K^+. Where indicated, cells were pretreated with Tris (120 mM) to permeabilize the outer membrane to valinomycin. **b**, Inhibition of FlgM secretion by acetate (34 mM) at pH 5.

downregulation of the master regulators *flhDC* (<3 μg ml^{-1}) (Table 1). Furthermore, the MIC value of the Δ*fliHIJ* strain was greatly increased (to 800 μg ml^{-1}) by overexpression of FliR, a membrane-associated part of the export apparatus (Table 1, and Supplementary Data). Prompted by this evidence of export in the absence of FliI, we examined motility of Δ*fliHIJ* cells. The Δ*fliHIJ* cells migrated in soft agar at about one-tenth the wild-type rate (Fig. 4a), and a fraction of the cells were observed to swim in liquid media. Cells isolated from the centre, edges or intermediate positions in the swarm showed the same phenotype when purified and re-tested (not shown), and so the slow motility is a property of the population and is not due to suppressing mutations. Staining showed flagella on a small fraction (<1%) of the cells (Fig. 4b, left panel). A Δ*fliI* mutant swarmed more slowly than the Δ*fliHIJ* strain (Fig. 4a) and also showed reduced export in the MIC assay (Table 1), consistent with the more severe motility defect reported previously for a Δ*fliI* mutant[4,17].

In addition to the flagella apparatus, members of *Salmonella* spp. contain two non-flagellar (injectisome) type III secretion systems, with associated ATPases InvC and SsaN[8,18]. To rule out any involvement of InvC or SsaN in the secretion observed in Δ*fliHIJ* cells, we repeated the experiments in a Δ*invC* Δ*ssaN* Δ*fliHIJ* strain. The triple-deletion mutant swarmed equally as well as the Δ*fliHIJ* strain in soft agar (Fig. 4a), and flagella were again seen on a few cells (Fig. 4b, right). Thus, none of the secretion ATPases is required for flagellar export, assembly or function.

Our conclusions are consistent with previous observations of a PMF requirement for flagellar growth from more than 25 years ago[19], and extend the earlier findings in showing that export can be energized by PMF alone in the absence of any type III secretion ATPase[7]. Use of the proton gradient is perhaps not surprising given the speed of type III secretion and the likely advantage of tapping a proximal energy source. Rapid subunit export presumably requires a rapid supply of energy, which might be more easily delivered by a proton current than by ATP hydrolysis. Given that type III secretion is energized by PMF, future studies should focus on the molecular mechanism of proton movement through the apparatus and its coupling to movement of substrate.

Table 1 | Effects of CCCP and mutation on flagellar export

Genotype (treatment)	MIC (μg ml^{-1})*
Δ*flgBC flgE:bla*	25
Δ*flgBC flgE:bla* (30 μM CCCP)	<3
Δ*flgBC flgE:bla* Tet-inducible *flhDC* (no Tet)	<3
Δ*flgBC flgE:bla* Tet-inducible *flhDC* (15 μg ml^{-1} Tet)	50
Δ*flgBC flgE:bla* Tet-inducible *flhDC*, Δ*fliF* (15 μg ml^{-1} Tet)	<3
Δ*flgBC flgE:bla* Δ*fliHIJ*	12
Δ*flgBC flgE:bla* Δ*fliHIJ*, *fliR* expressed from plasmid	800
Δ*flgBC flgE:bla* Δ*fliI*	6
Δ*flgBC flgE:bla* Δ*fliP*	4.4
Δ*flgBC flgE:bla* Δ*fliP*, *fliR* on plasmid	4.4
Δ*flgBC flgE:bla* Δ*fliP*, *fliP* on plasmid	800

* MIC, minimum inhibitory concentration of ampicillin required in cells with rod-gene (*flgBC*) deletions to direct the hook–β-lactamase (FlgE–Bla) fusion protein into the periplasm[13] (further details are provided in Supplementary Information).

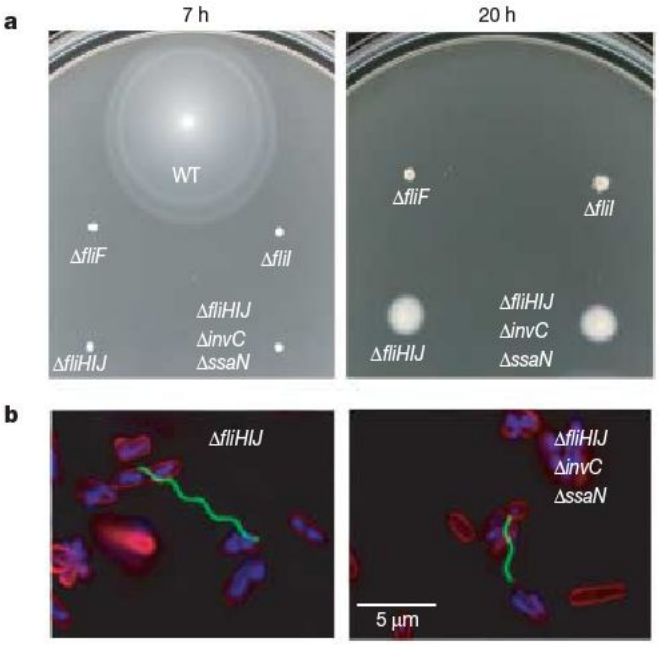

Figure 4 | FliI is non-essential for flagellar assembly and function. **a**, Swarming of the Δ*fliHIJ* deletion strain and a Δ*fliHIJ* Δ*invC* Δ*ssaN* strain with all type-III secretion ATPases deleted. The Δ*fliF* strain, blocked in the earliest step of flagellar assembly (Fig. 1), is included as a negative control. Plates were incubated at 32 °C. **b**, Flagella on cells of the Δ*fliHIJ* mutant (left panel) and the Δ*fliHIJ* Δ*invC* Δ*ssaN* triple-deletion (right panel). Flagella were visualized with FITC-conjugated anti-FliC antibody (green)[26]. DNA was stained with DAPI (blue), and membranes by FM64 (red).

METHODS SUMMARY

Media, growth conditions, methods for phage-mediated transduction and motility assays were performed as described previously[9,20,21]. Carbonylcyanide *m*-chlorophenylhydrozone (CCCP) and valinomycin were from Sigma (analytical grade). Potassium acetate was from J. T. Baker, growth media from Difco, and buffers from Sigma.

FlgM secretion was assayed by accumulation of the protein in the culture medium, using procedures described previously[22] with minor modifications described in Methods. Cells at mid-log growth stage were treated with the PMF-discharging agents (CCCP or valinomycin) at the concentrations indicated in the figures, introduced from freshly prepared stocks. Following centrifugation to remove cells, supernatants were filtered and FlgM was detected by immunoblotting, essentially as described[23].

ATP was measured using the firefly luciferase assay and sample-processing procedures of ref. 24.

Ampicillin resistance in FlgE–Bla-exporting cells was assayed as described[13], with minor modifications described in Methods. Briefly, cells were diluted to D_{600} = 0.01, cultured for 4.5 h in LB media containing ampicillin in a series of dilutions ranging from 800 μg ml^{-1} to 1.56 μg ml^{-1}, and the minimum inhibitory concentration (MIC) was taken as the lowest ampicillin concentration giving D_{600} < 0.05. The periplasmic fractionation and anti-Bla immunoblot detection of FlgE/Bla in the cellular and periplasmic fractions was performed as described by ref. 25.

Swarming motility was assayed in plates containing tryptone broth and 0.28% bacto-agar. Plates were incubated at 32 °C. Flagellar immunostaining used the methods of ref. 26.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Global competition for talent in science and engineering is increasing, according to the 2008 Science and Education Indicators released by the US National Science Foundation last week (www.nsf.gov/publications). And visas are a significant weapon in the battle to secure top researchers. "Many countries are actively reducing barriers to high-skilled immigrants entering their labour markets," the report says, while noting that "at the same time entry into the United States is becoming somewhat more difficult".

Canada and Japan, for example, have dramatically increased the number of visas they offer to skilled workers. In 2005, Canada issued 44,000 temporary visas, a 63% increase since 1995. Japan has been even more aggressive, with 268,000 workers entering on temporary visas in 2003, a 93% increase from 1992. Meanwhile, in the United States, similar visas have become harder to come by. In October 2003, the maximum number of available H-1B visas, often given to research and development (R&D) workers in information technology and other R&D fields, fell from 195,000 to 65,000. Apart from making it harder for US-based companies to hire foreign workers, this also discourages foreign students from staying in the United States to job hunt after they have finished their degrees; both Canada and Japan allow for more open-ended, longer-term residency on their temporary visas.

The visa situation sheds light on more than just immigration issues. The trend in countries such as Canada and Japan in part reflects investment in both infrastructure and R&D. For jobseekers, improvements in visa availability indicate where new opportunities are arising. In Japan, for example, much of the increase in immigration is from other Asian countries. Before the Japanese visa boost, those workers would probably have gone to the United States or Europe.

Jobseekers can view the visas available as lights illuminating doors, or jobs, in a country and the actual visa as the key to get in. Competing countries might find that one way to make gains in the battle for talent is to start deploying welcome mats.

Paul Smaglik is former editor of *Naturejobs*.

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JOBS OF THE WEEK

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Columbia, MO (USA)
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Argentina's pivotal moment

Rebuffing a troubled economic and political past, Argentina is trying to get on the science map with a new science ministry and attempts to retain young talent. **Paul Smaglik** reports.

CORBIS

Martin Giurfa struggled to survive as a scientist in 1980s Argentina. Under the country's military dictatorship, undergraduate biology training consisted of only botany and zoology. Evolution and neuroscience weren't in the curriculum. Political conditions made day-to-day living hard, let alone attempts at focusing on studying science. "That was a real nightmare in terms of persecution, terror and lack of intelligence," Giurfa says.

Threats of kidnapping abounded, and a number of people disappeared. Many senior scientists fled. When democracy returned in 1983, so did many scientists. "There was a huge explosion — many of the Argentinian professors in other countries came back," Giurfa says. "It was a fantastic time." His graduate education flourished as a result.

After three years of euphoria, the government in 1986 launched an economic plan that slashed funds for science and education. As the economy was hit by currency crises, research funding dwindled. "Most left. Some stayed," says Giurfa. "Those are the guys who deserve special credit." After a currency crisis emptied the government coffers, Giurfa had had enough. "I remember having to spend two to three months without salary, because the government didn't have enough money," says Giurfa, who left for Europe in 1990 and is now a professor of neuroscience at the University of Toulouse in France.

Another financial and political crisis struck in 2001, followed by a currency crisis in 2002. But after five years of relative stability, Giurfa, like many Argentinian scientists, thinks the tide is turning again. He expects a better future for the hundreds of scientists who have stayed and the hundreds who may be thinking of returning.

First science minister

President Cristina Elisabet Fernández de Kirchner — elected last year to succeed her husband Néstor Kirchner — has appointed molecular biologist Lino Barañao as the country's first-ever science minister. Argentinian scientists are excited because Barañao has spent much of his scientific career in the country. He has grappled with the challenges facing scientists trying to stay competitive, as both a university researcher and a member of the government.

These challenges are considerable. Scientists' salaries are low, even by Latin America standards. Equipment such as chemicals and reagents cost about twice the US price, and delivery is often delayed by trade restrictions. Although several large multinational drug and agricultural firms have a presence in Argentina, their investment in the country's research and development (R&D) has been negligible.

But the creation of the new ministry, and the selection of a working scientist to fill it, has given many scientists in Argentina a sense of cautious optimism.

"The feeling in the scientific community is a good one, not just because of the creation of this ministry, but particularly because the head is a scientist himself," says Marcelo Rubinstein, an investigator at the National Council for Science and Technology's institute for research on genetic engineering and molecular biology and professor at the University of Buenos Aires. "The whole nation, not only scientists, took the creation of a new ministry of a science as an indication of a more modern state."

But it will take more than a new ministry to make Argentina an attractive place to do science. Low pay is a major obstacle. "Without competitive salaries in science, the impact of scientific endeavours to improve Argentina's competitiveness is at a serious risk," says Rubinstein, who has won a series of Howard Hughes Medical Institute (HHMI) international scholarships.

Rubinstein has been able to pay his nine-member

Bright future: the Pierre Auger Observatory (above) and a traditional Buenos Aires street (top).

team relatively well, thanks to his HHMI funds. But such international support is not common in Argentina. Scientists are state employees, and Rubinstein is not optimistic that the government will soon be able to increase their salaries. Giving a pay rise to researchers but not to other federal employees would be politically unpopular. "I think the government will be very hesitant to admit that this is a critical point," he says.

Aiming high

Barañao, who recalls making $50 a month during one of Argentina's economic crises, is well aware that salaries need to be higher. He will encourage the government to increase overall investment in science — its current goal is 1% of GDP. "If we do that, we will be able to increase salaries," Barañao says, while emphasizing that the country's current standard of living and quality of life are not that bad. "The cost of living is cheaper here than in Brazil, for instance, he says. "It's amazing how the situation has changed."

Alberto Kornblihtt, professor of molecular and cell biology at the University of Buenos Aires, hopes that the new ministry will mean a science-budget increase, because the country needs infrastructure as well as higher salaries. "We need more new buildings and equipment," Kornblihtt says. He has in the past proposed taxes on multinational companies operating in Argentina to add to government funding for such facilities. Kornblihtt says several large multinationals have a sales presence, but invest little in terms of R&D or employing high-tech workers. He will encourage Barañao to change that situation. Multinationals should invest or spend a percentage in the local R&D economy, he says, or else pay a tax. "Otherwise they are just exploiting our country," he says.

Barañao says that, since the ministry's creation, two international drug companies have already expressed interest in Argentinian R&D. He wants to encourage more, as the country has well-trained scientists and an inexpensive workforce. "There are some tax incentives for biotechnology that we are considering, as well as for software and nanotechnology," he says.

Increasing confidence

Until that money materializes, young scientists who return are relying on the goodwill of colleagues in the country and hoping that investment and infrastructure will improve. Alejandro Colman-Lerner, a systems biologist at the University of Buenos Aires, has confidence in both. The political and economic climate has changed sufficiently to make his return to Argentina professionally viable. "If I'd have thought about coming back in 2001, I wouldn't have come back," says Colman-Lerner.

Even so, his return from the Molecular Science Institute in Berkeley, California, in 2005 wasn't easy. "There was no start-up funding," Colman-Lerner says. "I had to come back with basically nothing. The place where we are is not very big. My lab space is very limited." In California, he had ample space and equipment, as well as interactions with luminaries such as Sydney Brenner and Roger Brent, who trained him in molecular endocrinology and yeast genetics.

In Argentina, he also had to contend with a scientific culture that was unfamiliar with his budding interest in multidisciplinary work. "I didn't know if the physics people in Argentina would be interested," he says. He has managed to start collaborations with mathematicians and physicists, but now faces another frustration: the expense of chemicals and reagents. This is especially problematic given the limited start-up package for lab equipment, typically around US$35,000–40,000, which is about a third of the start-up cost in a US lab. "You have to pay shipping, insurance and a little bit more to get the goods out of

Will Lino Barañao's appointment as minister help scientists such as Pablo Bauleo (right)?

customs and you often have to pay a third party," says Colman-Lerner. As a result, many reagents cost 50–100% more than they would if purchased in the United States.

But expense isn't the most crucial sticking point. Delays in getting supplies mean that simple experiments have to be put off, and scientists have to be less nimble in trying different experimental approaches. In the United States, the time between thinking about an experiment and doing it is relatively short, Colman-Lerner notes. "In Argentina, it takes much more planning," he says. Barañao says he intends to negotiate prices with the suppliers and examine the import laws.

Despite the obstacles, Guillermo Lanuza, currently a research associate at the Salk Institute for Biological Studies in La Jolla, California, says he is looking forward to returning to Argentina in a month to start up a neurobiology lab at the Leloir Institute Foundation in Buenos Aires. He made the decision to return before the creation of a science ministry, but thinks it may encourage others to come back as well.

The situation in Argentina has slowly improved during the past few years, Lanuza says, with regular, albeit limited, amounts of start-up funds becoming available. "The funding situation is still very limited, but at least after several rounds of funding, the grants are now being financed on a regular basis," Lanuza says.

What Argentinian scientists need even more than a windfall for salaries and infrastructure, though, is some stability and a long-range plan. Science has risen and fallen along with the economy and politics during the past three turbulent decades. "Maybe the creation of this ministry will lay the basis for having a long-term policy, independent of who is in the government," Lanuza says.

Don't get too excited

A plan that steadily increases funding, boosts salaries and provides those who return with ample start-up funds could encourage researchers back from abroad. "A growing number of people are trying to return," Lanuza says. "At the same time we are very cautious, because history shows we should not be too excited."

Barañao wants to nurture stability. The economy has grown 8–9% a year during the past few years, he notes. "The future looks, I won't say bright, but more stable," he says. Once the government demonstrates increasing investments in science — and perhaps, creates some high-tech industry — more scientists will drop their caution about returning, Barañao says.

Javier Guillermo Magadán, a postdoc at the US National Institutes of Health, is monitoring the situation. He arrived in the United States in April for a two-year fellowship funded by the charitable Pew Foundation. His fellowship includes $35,000 in start-up funding, should he return to Argentina. The Argentinian government has established a similar scheme, with 120 expatriates applying for return funding. But there's no word yet on how many will be successful or the amount of money they will receive.

PIERRE AUGER OBSERVATORY

Looking up: the Pierre Auger Observatory's lidar scans the skies.

Magadán's future is complicated, because his wife Valeria Zarelli will finish her PhD this September and is seeking a postdoc in the United States. Magadán realizes that finding permanent positions for both of them in the same US city will be difficult, and anticipates better odds in Argentina. "People know us, know our work," he says. "Here it is a little difficult."

Barañao says that the country has secured an $80-million loan from the Inter-American Development Bank to repatriate scientists. "We will focus on young scientists finishing their postdoc training. We will provide money for tickets, travel, grants for initial development of the lab," he says. "And we will provide some salary supplement to make up the gap in income." So far, 130 people have applied.

Star collaborators

International funding has also helped with some of the country's scientific infrastructure. For example, 17 countries including the United States, France, Germany, Britain and Argentina (www.auger.org/collaboration) have pooled $50 million to build the Pierre Auger Observatory, a cosmic-ray telescope to be located in Mendoza, east of the Andes. That investment has provided training and jobs for some Argentinian scientists, but the pay-off has been slow, says Pablo Bauleo, currently an astrophysics postdoc at Colorado State University, Fort Collins.

When he was a graduate student, the observatory hadn't been approved and there were few astrophysics positions open. Now, he travels to the observatory four times a year to perform calibration and maintenance on the equipment and also make some observations.

Initially, scientists from the developed world looked at the Argentinians as muscle for "tightening up the screws", Bauleo says. Now some of the postdocs and students who helped get the observatory running are finding work elsewhere. Bauleo hopes his work there will help him land a permanent position, perhaps at the northern arm of the observatory to be built in Colorado.

He is less optimistic about things in Argentina improving to the point where he could land a stable position. "The track record is not so good," Bauleo says.

But if Barañao can summon the resources, and if Argentina can break out of economic and political strife, the country's scientists stand to benefit. "It's a country that's been on a rollercoaster," says Rubinstein. "Anything can happen." But having a chief scientist means that the rollercoaster finally has the potential for a plan — and accountability.

"I am very optimistic," says. Kornblihtt. "Lino Barañao is well-prepared to lead us. Nevertheless, if things don't go right, I will be the first to criticize." ■

Paul Smaglik is a freelance writer in Milwaukee, Wisconsin.

Optimistic: Martin Giurfa (top) and Marcelo Rubinstein.

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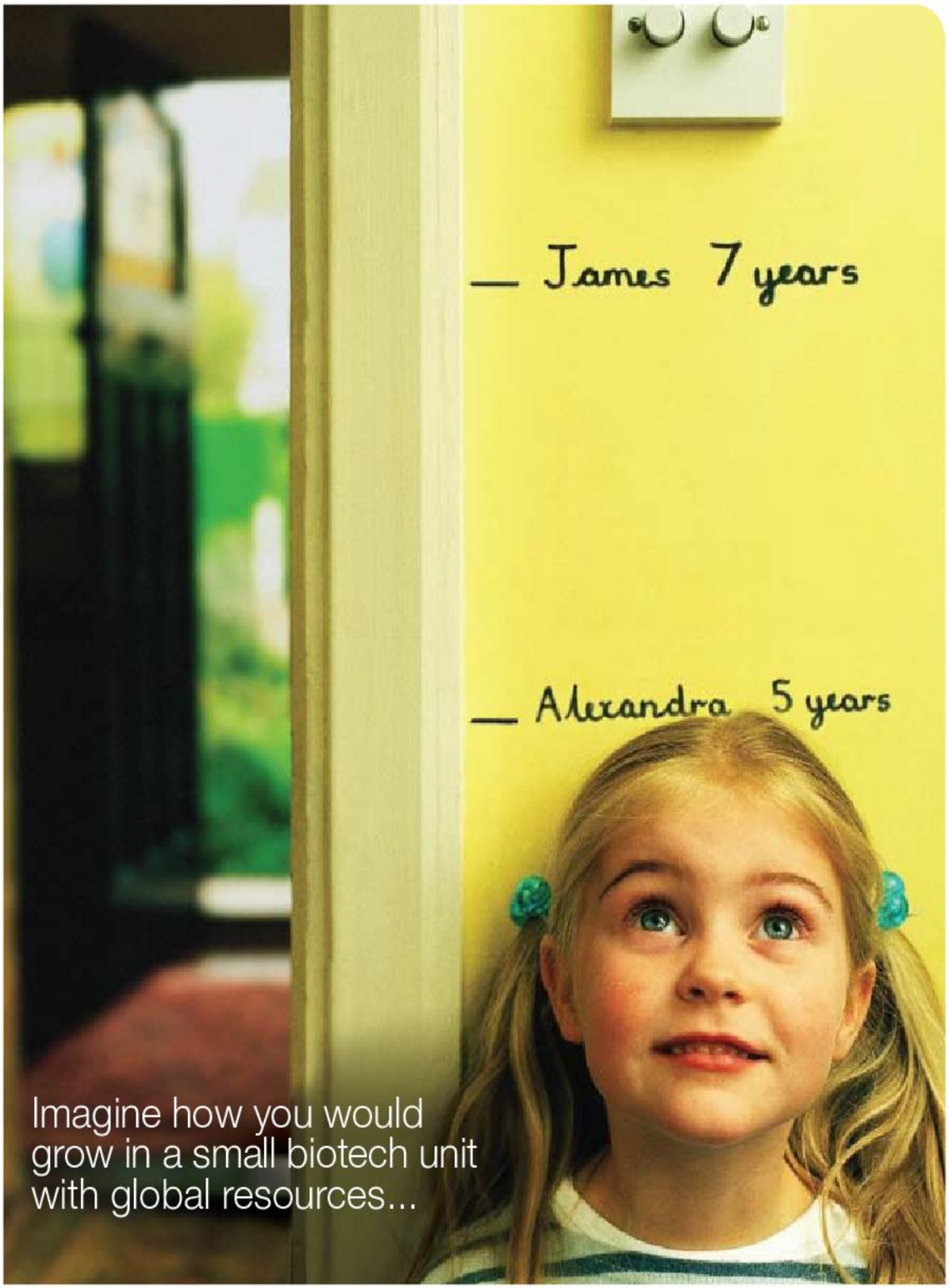

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Prof. Dr. Michael Kühl (email: michael.kuehl@uni-ulm.de) or
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The Havana team is responsible for the analysis and annotation of genomic sequence. As part of the Havana team you will be involved in analysing and interpreting human and vertebrate sequence data. This will include preparing and maintaining sequence annotation information and primary publications for the public sequence database. You will also work alongside software and database developers to enhance annotation tools and assess the automatic analysis procedure. A PhD in biology, molecular biology or related field would be preferred, however extensive research experience in genomics or genetics may be considered. Good communication skills and a keen eye for detail are essential. These positions provide an excellent opportunity to use molecular biology experience in a non-laboratory field.

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Medical Biomics Centre

Medical Biomics Genomics Scientist

We have an exciting vacancy for a Postdoctoral Scientist (Genomics) appointed jointly between the Medical Biomics Centre (MBC) and the Centre for Medical Genetics. Time will be split equally between research on specific genetics project(s) within Clinical Development Sciences and as a senior member of the MBC Team collaborating with other SGUL researchers. You will apply a range of modern genomic/transcriptomic research techniques centred on our new Illumina BeadStation. There will also be the opportunity to develop collaborations with researchers employing a wide-range of integrated genomic and proteomic strategies in the MBC.

Applicants should have a PhD. with proven understanding of modern genomic/transcriptomic strategies and practical skills in relevant technologies.

Starting salary will be in the range £30,030 - £35,360 pa inclusive.

Please quote reference number: 101/08.

Closing date: 21 February 2008.

Division of Basic Medical Sciences

Postdoctoral Research Assistant

Fixed-term (3 years)

Applications are invited for a Postdoctoral Molecular Biologist preferably with experience of ion channels to be involved in a British Heart Foundation funded project. The grant aim is to investigate the molecular identity of calcium-activated chloride channels in vascular smooth muscle cells and will be directed and supervised by Dr Iain Greenwood and co-holder Dr Debbie Baines.

You must be experienced in a variety of molecular techniques including qRT-PCR, cloning, western blot, heterologous expression and RNAi. You will also be expected to contribute to the development of research ideas directed towards improving our basic understanding of smooth muscle function. This will include undertaking pilot studies for future grant applications if necessary. There will be the opportunity to gain supervisory experience.

Starting salary up to £30,030 pa (inclusive of London allowance)

Please quote reference number: 102/08.

Closing date: 7 February 2008.

Division of Cellular & Molecular Medicine
Centre for Infection

Research Assistant

This position is within Professor Robin Shattock's research group working on HIV prevention strategies including microbicides and vaccines as part of the Immunology Core Team. The project is specifically aimed at inducing broadly reactive neutralising antibodies against HIV as part of a Europe wide network. You will provide critical data and develop new assays in support of novel HIV vaccine development programmes aimed at inducing protective immunity.

You must be responsive to changing requirements in this fast moving field and have experience in working with clinical materials and immunoassays. Knowledge of Good Clinical Laboratory Practice is desirable and the ability to work to deadlines.

Salary for the post will be in the range of £24,246 - £29,230 pa inclusive.

Please quote reference: 300/08.

Closing date: 7 February 2008.

For further information and an application form, visit or contact the Recruitment Team on 020 8725 5020 (24-hour answerphone) or email: personnel@sgul.ac.uk

Please quote the appropriate reference number.

St George's is an Equal Opportunities Employer

U123171R

U122928RL

Wellcome Trust Sanger Institute
Mapping Group
Group Leader
The Mapping Core Group represents a consolidation of existing technologies and clone resources for mapping. You will have full line management responsibility and will communicate with external collaborators We are looking for a highly motivated individual with excellent interpersonal skills. A science degree and experience of people management essential.

Contact

Human Resources
recruit@sanger.ac.uk
www.sanger.ac.uk

W123023RL

University of Ulm
Stem Cells, Aging and Cancer
Postdoctoral Position
Postdoctoral Position at the Max-Planck-Research Group for Stem Cell Aging.
The applicant should have an outstanding track record on ES-cell work, microinjection generation and characterization of knockout mouse models, stem cell work.
Send application to: Lenhard Rudolph, Department of Molecular Medicine, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Contact

Prof. Lenhard Rudolph
Email: Lenhard.Rudolph@uni-ulm.de

W122646RL

Novo Nordisk
R&D-Protein Engineering
Postdoctoral Research
Position is available in analytical protein chemistry for a period of three years.
The Department is engaged in discovery activities directed towards the development of new insulin and GLP-1 analogues and formulations for improved treatment of diabetes as well as purification. You hold a Ph.D. Deadline: 25th feb. 2008.
REF: NN37588, Postdoctoral.

Contact

Peter Kresten Nielsen
Tel: +45 3079 0375
http://www.novonordisk.com/

Eli Lilly and Company is a world leading pharmaceutical organisation. We are a premier provider in the discovery, development, manufacturing and marketing of a broad range of pharmaceutical products. Based on our continuing success we have the following roles:

POST-DOCTORAL FELLOWSHIP PROGRAMME IN COGNITIVE NEUROSCIENCE

The Lilly Centre for Cognitive Neuroscience is a consortium of academic and industrial neuroscientists seeking to enhance the probability of clinical success for molecules targeted at conditions involving cognitive dysfunction.

In order to further these aims Lilly Research Laboratories have appointed a number of Post-doctoral Fellows working with leading neuroscientists from the Universities of Cambridge, Oxford, Bristol and Cardiff based at the Lilly Research Centre, Surrey, UK. The Fellows are aligned to one or more industrial co-supervisors from the Discovery Biology division of Eli Lilly and Co Ltd. The Centre now seeks to recruit:

A behavioural neuroscientist with interests in preclinical models of cognitive impairment in schizophrenia to work on neurodevelopmental aspects of the disease alongside colleagues specialising in the investigation of neuranatomical and electrophysiological consequences of developmental challenges (Professor Trevor Robbins, University of Cambridge and Professor Simon Killcross, Cardiff University, Reference: CCN08/004).

The successful candidate will have a PhD in preclinical cognitive neuroscience, will have demonstrated potential for leadership in their areas of expertise and be committed to applying their skills to furthering our knowledge of the psychopharmacology of cognition. The position requires good oral and written communication skills and offers a unique opportunity to carry out basic scientific research within a cutting-edge, highly motivated, productive, yet supportive, state-of-the-art industrial environment closely linked to academic centres of excellence.

A letter of application describing why you think you might be able to fulfil the above conditions, together with a full CV should be sent as soon as possible to:

Personnel Selection On-Site Office, Lilly Research Centre, Erl Wood Manor, Windlesham, Surrey GU20 6PH, UK or E-mail: Personnel_selection@lilly.com

Please quote the reference in all correspondence.

www.lilly.co.uk

Lilly
Answers That Matter.

W123077R

nature

Business Development Executive

Nature is the world's leading weekly scientific journal and is the flagship publication of Nature Publishing Group.

We are now looking for a full-time Business Development Executive to work on Nature special sales both in print and online.

The successful candidate will lead Nature's special sales and business development, including but not restricted to Nature Insights, Nature Outlooks and online initiatives and will be expected to generate and lead new publishing projects both in print and online. General publishing knowledge is desirable whilst a business development/sales background is essential. The position would especially suit those with a business development background who wish to learn more about publishing, and use their sales skills to develop publishing initiatives which generate both editorial and commercial impact.

Candidates should be confident, self starters, able to work independently, and have a proven track record in revenue generation and business development. They should also have a keen interest in scientific communication, and publishing in general. Candidates should have an analytical approach to problem solving and a keen understanding of project management. Strong interpersonal skills and a customer service ethos are essential.

The position will be full-time.

The position is based in our modern London offices.

Please send your CV, a summary of relevant experience, and your current salary, quoting reference number to NPG/LON/812, to Geetika Juneja Personnel Assistant at londonpersonnel@macmillan.co.uk

All candidates must demonstrate the right to live and work in the UK to be considered for the vacancy.

Closing date: 7th February 2008

nature publishing group npg

IN123102R

UCL

UCL The MRC Laboratory for Molecular Cell Biology

Postdoctoral Training Fellow

The MRC Laboratory for Molecular Cell Biology is an internationally renowned Molecular and Cell Biology Institute situated at University College London (http://www.ucl.ac.uk/lmcb)

We are seeking a highly motivated and creative Scientist to join the team led by Dr Alison Lloyd, to study novel mechanisms of tumour development in Neurofibromatosis Type 1 (EMBO J (2004) 23, 3061-3071, Cell Cycle (2004) 3 (10) e69-672, Science (2001) 291, 872-875). For more information about the research topic please visit:

http://www.ucl.ac.uk/lmcb/research-groups/lloyd.htm

Candidates should possess an MD and/or a PhD degree in a relevant Biomedical discipline. Experience in Cell Biology is preferred, with a background in Cancer Biology an advantage.

Available in April 2008, the post is funded on a fixed-term contract for up to three years' in the first instance. The starting salary will be on Grade 7, from £29,238 to £35,368 inclusive of London Allowance, depending on previous experience.

Applications, including two copies of your CV, the names, postal and e-mail addresses of two referees and a completed Equal Opportunities Classification form (available at www.ucl.ac.uk/hr/jobs/), should be sent to:

Ione Karney, MRC Laboratory for Molecular Cell Biology, UCL, Gower Street, London WC1E 6BT UK (email: i.karney@ucl.ac.uk)

Closing date: 29th February 2008.

UCL Taking Action For Equality.

U123208R

Freie Universität Berlin

With the 2006 and 2007 decisions in the Excellence Initiative by the German federal and state governments to promote science and research at German universities, Freie Universität Berlin was granted funding for five Graduate Schools, three Clusters of Excellence, as well as for its institutional strategy to promote top-level research - a concept called "Freie Universität Berlin - An International Network University". Freie Universität Berlin, consequently one of the most successful universities in Germany, interprets this selection made by a joint commission of representatives from the German Research Foundation and the German Science Council as a mandate to continue on its path of strategic international networking by maintaining outstanding research clusters and excellent graduate programs. It will thus have a crucial role in securing the future of the Berlin-Brandenburg area as a beacon of education and research. In accordance with the newly established Clusters of Excellence and Graduate Schools, applications are invited for the following positions:

Cluster of Excellence "Languages of Emotion"

Department of Educational Science and Psychology
Institute of Psychology

invites applications for a tenured

Professorship in Psychology of Emotions (W3)

The successful applicant will be required to provide teaching and research in this area.
For the initial duration of four semesters, the teaching load will be reduced to 50 percent of the regular teaching load.

In line with article 100 of the Berlin Higher Education Act *(Berliner Hochschulgesetz)*, a *Habilitation*, junior professorship, or equivalent postdoctoral qualification is required for this position.

The successful candidate will hold a doctorate in Psychology or Cognitive and Affective Neuroscience, with a broad focus on multiple fields and methods of Emotional Psychology reflected by ample international research and teaching activities in the aforementioned area. Candidates are further expected to have a proven record of publications in internationally renowned journals, as well as experience with securing and implementing externally funded research projects.

Active involvement in the interdisciplinary "excellence cluster" *Languages of Emotion* is expected through research aimed at the development of new models and approaches for issues in the study of emotions - in particular of neurocognitive methods such as electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). These methods are to be applied to interdisciplinary matters of the following research areas: speech processing and language acquisition; nonverbal communication; esthetic perception; and emotional regulation.

In general, the language of instruction will be German, but some teaching may be conducted in English. A non-German-speaking appointee will be expected to be able to teach in German within two years.
The vacancy is a W3-type position with tenure. The appointee will be granted either civil servant or comparable salaried employee status.

Freie Universität Berlin is an equal opportunity employer.

Applications quoting reference **#12-84/07** must be received at the address below by **February, 21st 2008** and should include a letter of motivation describing your interest in the position and pertinent experience, a curriculum vitae including three relevant references, a list of publications, as well as copies of the certificates of all academic qualifications held.

Freie Universität Berlin
Fachbereich Erziehungswissenschaft und Psychologie
Dekanat
Postfach 1
Habelschwerdter Allee 45
14195 Berlin (Dahlem), Germany

Department of Educational Science and Psychology
Institute of Psychology

invites applications for a

Junior Professorship in Evolutionary Psychology

The successful applicant will be required to provide teaching and research in this area.
For the initial duration of four semesters, the teaching load will be reduced to 50 percent of the regular teaching load.

In line with article 102a of the Berlin Higher Education Act *(Berliner Hochschulgesetz)*, the successful candidate will have a Ph.D. reflecting her or his outstanding academic qualification. Previous work in the academic field - both prior and subsequent to the conferral of the doctoral degree - should not exceed a total of six years.

The appointee will hold a doctorate in Evolutionary Psychology or Evolutionary Biology, with an exceptional background of research activity in that field, preferably in an international context. She or he will have a proven record of successful university teaching and publications in internationally renowned journals. Candidates are further expected to have experience with externally funded research.

Active involvement is expected in the "excellence cluster" *Languages of Emotion* through research aimed at the development of interdisciplinary approaches to issues in Evolutionary Psychology or Evolutionary Biology such as: speech processing and language acquisition; nonverbal communication; esthetic perception; and emotional regulation.

In general, the language of instruction will be German, but some teaching may be conducted in English. A non-German-speaking appointee will be expected to be able to teach in German within two years.

The initial contract for this position is for the duration of three years, for which the junior professor will be granted civil servant status with limited tenure (W1). Provided that her or his performance is evaluated positively, the contract will be renewed for three more years.

Freie Universität Berlin is an equal opportunity employer.

Applications quoting reference **#12-83/07**must be received at the address below by **February, 21st 2008** and should include a letter of motivation describing your interest in the position and pertinent experience, a curriculum vitae including three relevant references, a list of publications, as well as copies of the certificates of all academic qualifications held.

Freie Universität Berlin
Fachbereich Erziehungswissenschaft und Psychologie
Dekanat
Postfach 1
Habelschwerdter Allee 45
14195 Berlin (Dahlem), Germany

W122878R

Freie Universität Berlin is a state-funded university. It has some 35,000 students and 350 professors, medical institutions not included.
The university has 11 departments structured into more than 70 institutes, plus the Charité medical center, which is made up of the former medical schools of Freie Universität and Humboldt Universität. Detailed information is available at **www.fu-berlin.de** and **www.ewi-psy.fu-berlin.de**.

CRISP: Combinatorial Responses In Stress Pathways

University of Aberdeen and Imperial College London are seeking ambitious and committed scientists to join the CRISP team. This large, strong, interdisciplinary team will include four experimental biologists, four applied mathematicians and six PhD students in addition to the ten group leaders. We are studying the Systems Biology of stress responses in the medically important fungi *Candida albicans* and *Candida glabrata*. We combine advanced modelling techniques with state-of-the-art genomics, molecular biology, biochemistry and cell biology in these pathogens. This exciting project is funded by the BBSRC under the SABR initiative for up to five years.

Informal enquiries may be made to Professor Al Brown (al.brown@abdn.ac.uk) or Dr Ken Haynes (k.haynes@imperial.ac.uk).

University of Aberdeen positions: **Online application forms and further particulars are available from www.abdn.ac.uk/jobs or telephone (01224) 272727 (24-hour answering service) for an application pack.**

Imperial College London positions: **Online application forms and further particulars are available from http://www3.imperial.ac.uk/employment, or telephone 020 8383 4575 for an application pack.**

In all cases please quote the relevant reference number.

Studentships: **Applicants for studentships should follow the instructions below.**

Closing date: 22 February 2008.

University of Aberdeen

Postdoctoral Fellow

You will be responsible for the development of quantitative models that predict the molecular behaviour of *Candida* to specific stress conditions. You must have experience of mathematical modelling of biological systems at the cellular level, including numerical simulation of biochemical pathways. Experience of modelling fungal stress responses and knowledge of the theory of dynamical systems or stochastic processes is desirable.

Salary will be in the range of £27,857 – £29,553 per annum. **Ref: YMB144R.**

Postdoctoral Fellow

You will be responsible for the development of methods to learn and construct qualitative models of stress signalling responses in *Candida*. You should have experience in Qualitative Reasoning and Machine Learning.

Salary will be in the range of £27,857 – £29,553 per annum. **Ref: YMB145R.**

Two Postdoctoral Fellows

You will be responsible for the genomic, molecular and biochemical experiments in *C. albicans* that underpin the stress modelling activities of the team. Post 1: You must have experience of (fungal) microarrays and molecular biology. Post 2: You will have experience of (fungal) biochemistry and cell biology.

Salary will be in the range of £27,857 – £29,553 per annum.
Ref: YMB146R (Post 1) or YMB147R (Post 2).

All the above posts are available for up to five years initially.

Two PhD students

Two four-year PhD studentships are available at University of Aberdeen to start in October 2008. You will be integral members of CRISP. Applications for the following projects should be made through the Graduate School www.abdn.ac.uk/clsm/graduate

1. Modelling stress responses in fungal pathogens.
2. Regulation of nitrosative stress responses in the major fungal pathogen of humans, *C. albicans*.

The first project will be supervised by the Grebogi-Moura-Thiel-Romano team, and the second by Brown and Gow. Both will also have cross-disciplinary co-supervisors from Imperial College.

1 4 9 5 UNIVERSITY OF ABERDEEN

Imperial College London

Postdoctoral Fellow

Responsible for the genomic, molecular and biochemical experiments in *C. glabrata*, you will supervise on a day to day basis the research technician. You must have experience of molecular biology, microarray experimentation and analysis, cell biology and/or biochemistry. Preferably in a fungal or stress analysis system.

Salary will be in the range of £24,570 – £28,820 per annum. **Ref: HM2008002.**

Research Technician

You will be responsible for technical support to the experimental CRISP team at Imperial and have experience of a biological sciences research laboratory.

Salary will be in the range of £21,560 – £23,930 per annum. **Ref: HM2008003.**

Four PhD students

Four four-year PhD studentships are available at Imperial College London to start in October 2008. You will be integral members of CRISP. All studentships will be at the interface of biology and modelling and applicants from all appropriate backgrounds (e.g. biology, mathematics, computing, physics, engineering, chemistry) may apply. Sample PhD projects include:

1. Prediction and verification of a protein-protein interaction network in *C. glabrata*.
2. Modelling Hog1 activation in *C. albicans* and *C. glabrata*.
3. Prediction of conditionally lethal nodes in the stress transcriptional network.
4. Comparative and evolutionary analysis of stress responses in fungi.

All students will have co-supervisors from Aberdeen University. BBSRC eligibility criteria apply.

To apply for these studentships e-mail a CV, personal statement and the details of two academic referees to Dr Ken Haynes (k.haynes@imperial.ac.uk).

Promoting Diversity and Equal Opportunities throughout both Universities

Imperial College London

U123221R

EMBL

EMBL-EBI

Part of the European Molecular Biology Laboratory (EMBL), the European Bioinformatics Institute (EBI) is located on the Wellcome Trust Genome Campus at Hinxton, near Cambridge and provides cutting-edge research, service and training in the field of bioinformatics. The EBI, a vibrant and multicultural science institute, is looking for a

Team Leader – Literature Service

As a global centre for Molecular Biology Data the EBI hosts large databases of biomolecular information and offers comprehensive services over the World Wide Web. The EBI provides access to the electronic literature both as stand-alone services and in combination with its other databases. Basic research in text mining accompanies these service activities and stimulates their enhancement. We seek to appoint a Team Leader for a small team which will enhance and bring focus to our literature-related services. The post holder must be able to lead a team and work in collaboration with other groups both within EMBL-EBI and other organisations.

This is a technical post, requiring an understanding of the computational, storage, and network methodologies appropriate to the task. It would suit a candidate with substantial experience in electronic information provision, and an interest in text and literature services. This experience may have been gained, for example, in a publishing, scientific or information-services context. Candidates are likely to be graduates in a computationally rich discipline, with significant practical post-graduate experience. Ideally (s)he will have understanding and experience in writing applications for funding.

An initial contract of 3 years will be offered to the successful candidate. This may be renewed, depending on circumstances at the time of review.

EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

The closing date for applications is March 24. Interviews will be held on May 13, 2008.

To apply, please send a CV, three references (not names of referees), and an outline of how you would provide leadership to the group, by email, quoting ref. no. N/08/003/EBI in the subject line, to:
applications@ebi.ac.uk

www.embl.org
www.ebi.ac.uk

W123037R

The University of Edinburgh

The University of Edinburgh is an exciting, vibrant, research-led academic community offering opportunities to work with leading international academics whose visions are shaping tomorrow's world.

Research Fellow (2)

£27,466 – £32,796

Post 1: Working with Justin Goodrich on the "Role of Polycomb-group genes in commitment to flowering in Arabidopsis", you will have a Phd and strong molecular biology skills. Experience in plant development and epigenetics is highly desirable.

Post 2: Working with Andrew Hudson, you will examine the genetic basis for local adaptation in Arabidopsis. A PhD and practical experience of at least one aspect of evolutionary genetics is essential.

These posts are fixed-term for three years.

Apply online, view further particulars or browse more jobs at our website. Alternatively, telephone the recruitment line on 0131 650 2511. Ref: 3008394NA (Post 1) or 3008499NA (Post 2). Closing date: 10 February 2008.

Committed to Equality and Diversity

www.jobs.ed.ac.uk

U123230R

One Postdoctoral position at Consorci Laboratori de Genètica Molecular Vegetal CSIC-IRTA

The Plant Molecular Genetics Laboratory CSIC-IRTA located in Barcelona, houses two Units, the Department of Molecular Genetics from the Institute of Molecular Biology of Barcelona from CSIC (Spanish Research Council, www.csic.es), and the Department of Plant Genetics from IRTA (Institute for Food and Agricultural Research and Technology, www.irta.es).
Applications are invited from individuals with recent Ph.D. degrees to join one group working in the Laboratory for one year renewable for a second one and starting from March 2008. A strong background in Molecular Biology and interests in Plant Biology are required.

Project: "Flower development"
The candidate will be involved in a project aimed to study floral development in Arabidopsis thaliana. The mail goal is to study the balance between promoting and repressing activities in order to trigger flowering. Different genetic, molecular and genomic approaches will be followed. Researcher responsible: Soraya Pelaz. sphgmh@ibmb.csic.es

Selected bibliography:
Pedro Robles and Soraya Pelaz (2005). Flower and fruit development in *Arabiodpsis thaliana*. International Journal of Developmental Biology. 49,633-643.
Cristina Castillejo, Maida Romera and Soraya Pelaz (2005). A new role of the *Arabidopsis SEPALLATA3* gene revealed by its constitutive expression. The Plant Journal. I43, 586-596.

Application: Candidates should send CV, publication list and names of three references through our web www.csic-irta.es indicating the project of interest and English skills.

Deadline February 10, 2008.

The Postdoctoral contracts are funded by a grant from the "Direcció General de Recerca, DIUE, Generalitat de Catalunya".

W122898R

Babraham Institute PhD Student Opportunities in Ageing Research 2008

Babraham Institute

The Babraham Institute is an international focus for innovative research in post-genomics studying gene function in cells, organs and systems, supported principally by the Research Councils. It is a recognised postgraduate teaching Department of the University of Cambridge. To support our new Institute Initiative in Ageing Research three BBSRC Targeted Priority Studentships will be available at Babraham, starting from October 2008, leading to a University of Cambridge PhD degree in the area of "Molecular and cellular mechanisms regulating ageing". Babraham Science is uniquely placed to make significant inroads into the fundamental molecular and cellular biology mechanisms that are responsible for the ageing phenotype. Babraham is a recognised centre of excellence in areas of signalling, genomic and epigenetic research, employing integrative in vivo (e.g. genetically/nutritionally modified), ex vivo (e.g. adult stem cell) and in vitro models. The three studentships can be awarded for up to four years and are in addition to our normal Quota Studentships allocated via our Open Day.

Eligibility for Funding: Please consult the following website (http://www.bbsrc.ac.uk/funding/training/eligibility.pdf) for details of eligibility and funding. Students will join a thriving scientific community situated on an attractive parkland campus near Cambridge. Our 70 students are all members of Cambridge Colleges and participate fully in University social and academic life (www.bio.cam.ac.uk/gradschool).

Details of our scientific programmes can be found on www.babraham.ac.uk. The Institute is fully equipped for state-of-the-art biological research including: innovative molecular biology, stem cell manipulation and transgenics, real-time laser scanning confocal microscopy, fluorescence sorting of cells, gene targeting and knockouts, mouse models of disease, mouse behavioural testing and proteomics. Selected students will be invited to attend interviews, discuss their research interests and view the Institute's facilities.

Potential projects are listed below and further details of each project are also given on the Student pages of our website (www.babraham.ac.uk); supervisors welcome informal enquiries:

Epigenetics of foetal programming
Dr Gavin Kelsey, gavin.kelsey@bbsrc.ac.uk

Foetal programming of cardiac health
Dr Llewelyn Roderick, llewelyn.roderick@bbsrc.ac.uk

Regulation of cardiac myocyte remodelling
Dr Martin Bootman, martin.bootman@bbsrc.ac.uk

Axonal transport of mitochondria during ageing
Dr Michael Coleman, michael.coleman@bbsrc.ac.uk

PI3Kinase and ageing-related changes in the immune system
Dr Klaus Okkenhaug, klaus.okkenhaug@bbsrc.ac.uk

The role of Akt in adult muscle stem cells
Dr Jenny Pell, jenny.pell@bbsrc.ac.uk

Autophagy and age-dependent regenerative potential of stem cells
Dr Nicholas Ktistakis, nicholas.ktistakis@bbsrc.ac.uk

Travel expenses will be paid to those invited for interview. Applicants should submit a full Curriculum Vitae with a covering letter indicating the two projects in which they are most interested, in order of preference, and ask two referees to write to the Institute on their behalf before the deadline.

Please send your applications to: Ms Linda Notton, Graduate Studies Programme, The Babraham Institute, Babraham, Cambridge CB22 3AT, Tel: 01223 496338, Fax: 01223 496022 or email babraham.graduate@bbsrc.ac.uk by **FRIDAY 15th FEBRUARY 2008**.

An Institute supported by the Biotechnology and Biological Sciences Research Council. An Equal opportunities employer.

www.babraham.ac.uk

U123229R

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

GGL International Giessen Graduate School for the Life Sciences

The **International Giessen Graduate School for the Life Sciences (GGL)** at the Justus Liebig University Giessen invites applicants for its

Doctoral Program in the Life Sciences

The Doctoral Program consists of a three-year interdisciplinary graduate course curriculum combined with an experimental project leading to a dissertation. Seminars and courses are conducted in English; German language courses are offered to international students. A Master´s degree or equivalent is required for admission. Applicants may choose between the following tracks: Man, Nutrition and Environment; Infection and Immunology; Heart, Lung and Blood Vessels; Biochemistry and Molecular Biology; Neurosciences; Reproduction in Man and Animals; Stress Resistance and Adaptation; Synthetic Molecules and Materials for the Life Sciences; Dental Medicine. Names of prospective supervisors and topics of research projects as well as other information necessary for application can be found on

http://www.uni-giessen.de/cms/fbz/zentren/ggl/application

Applicants should contact the listed supervisors directly for more information before submitting their application.
Your application should be sent to the following address **before February 22, 2008: Professor H. Michael Piper, MD PhD, Director of the GGL, Department of Physiology, Justus Liebig University, Aulweg 129, 35392 Giessen, Germany, Attn.: GGL 2008, Email: office@ggl.uni-giessen.de**

W123170R

UNIVERSITY OF BRADFORD
MAKING KNOWLEDGE WORK™

Confronting Inequality
Celebrating Diversity

Postdoctoral Research Associate in Proteomics

Ref: RCRU2301/NM

Grade 8, £29,139 - £32,796 p.a.

Fixed term for 3 years

Institute of Cancer Therapeutics

Join a team characterising proteins relevant to the development of anti-cancer agents. The Institute (RAE 5 rated) has an international reputation for translational research leading to the identification of novel therapies and additionally is part of an Experimental Cancer Medicine Centre (ECMC) to provide PKPD support to early phase clinical trials. The Institute is housed in a state-of-the-arts new building with dedicated facilities for medicinal chemistry, target validation, drug analysis, proteomics, tumour biology and pharmacology. For further information, please log onto www.cancer.brad.ac.uk

You will be responsible for the development of novel phospholipid-based affinity capture materials in a project funded by Bio-Rad Laboratories. The capture materials will be used to identify protein targets for drug development in cancer therapeutics using proteomics techniques. You will have practical knowledge of proteomics, HPLC, mass spectrometry and preferably experience of lipid chemistry and bioinformatics.

Informal enquiries prior to application can be made to Dr Chris Sutton, Institute of Cancer Therapeutics on 01274 236480 or by email: c.w.sutton@bradford.ac.uk

Applications from agencies will not be considered

Closing date: 11th February 2008

How to apply

jobs@bradford.ac.uk tel: 01274 233091
(minicom: 01274 235807)

www.bradford.ac.uk/jobs

INVESTOR IN PEOPLE

U123214R

GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN

The Faculty of Biology at the Georg-August-University of Göttingen and the German Primate Centre (DPZ) in Göttingen have established

a Professorship for Infection Biology

(W3 pay level)

at the Institute for Microbiology and Genetics, University of Göttingen, and within the Section for Infection Research at the German Primate Centre

The professor will head a research group at the DPZ. Correspondingly, the research activity will be located at the DPZ. The group should focus on primate-based research on infectious diseases affecting humans. Additionally, the professorship will contribute to the teaching programs of the Faculty of Biology.

Experience with experimental primate-based infection research is desirable and outstanding scientific accomplishments are expected. Representatives of the DPZ and its boards participate in the selection procedure and the application documents will be made available to them. A higher doctorate (Habilitation) or equivalent experience in teaching and research is required. The details are governed by the Higher Education Law of Lower Saxony (Nds. GVGl 2002 p. 286). The Foundation University of Goettingen holds the right for the appointment. Details will be provided on demand (Fax: +49 (0)551-39 2795; E-mail: dekanbio@gwdg.de) Applications of foreign scientists are specifically desired. Handicapped applicants with equivalent qualifications will be given priority. The University wishes to increase the proportion of women in academic positions and strongly encourages qualified women to apply. Women will be given precedence in all appointments in areas in which they are in a minority. Part-time employment can be enabled owing to circumstances.

Applications with supporting documents (curriculum vitae incl. publication record and description of teaching experience, copies of certificates, publication list plus the three most important full text publications and a research plan) should be submitted until the 06th of March 2008 to the dean of the Faculty of Biology, Untere Karspuele 1a, 37073 Goettingen.

W122796R

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www.cardiff.ac.uk/jobs

VTCT WORLD CLASS QUALIFICATIONS

CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

VTCT Professor of Burn Injury Study (Immunology)

School of Medicine

Cardiff University wishes to appoint a Professor of Immunology with an interest in burn injury, or a closely related area to provide leadership for the newly established Healing Foundation UK Centre for Burns Research. This, the first multidisciplinary centre of its kind, is generously funded by the Healing Foundation (www.thehealingfoundation.org) in partnership with Cardiff University, the Welsh Centre for Burns and Plastic Surgery and Swansea University.

The Centre will bring together basic and clinical researchers and clinicians to address three key areas of research in burns today: inflammation and infection, wound healing and rehabilitation. The goal is to deliver more rapid and effective methods of diagnosis and treatment in a world-class collaborative environment.

We are seeking to appoint an outstanding clinical or non-clinical scientist to the newly established VTCT Chair in Burn Injury Study. The Chair will be based in the School of Medicine within the Infection, Immunity and Inflammation-interdisciplinary research group (www.cardiff.ac.uk/medic/research//irgs/i3/) that co-ordinates immunology research in Cardiff University. You will be expected to lead an internationally competitive independent programme of research in immunology that relates to burn injury. The post will come with associated academic positions, start up funds and full-time administrative support. The VTCT Professor will be expected to take a lead role in the organisation of 4 year PhD and clinical research fellow schemes that are already funded within the program.

You should have an international reputation for your research in immunology, together with a track record in, or clear intent to develop, burn injury or a closely associated research area. You will be expected to work closely with clinical partners within the UK centre and provide the vision to further the goal of research translation, to support emerging clinical science programmes and therapy development.

Informal enquiries about the post can be made to Professor Nick Topley (topley@cardiff.ac.uk), Professor Tricia Price (Pricepe@cardiff.ac.uk) or Professor Paul Morgan (morganbp@cardiff.ac.uk).

Salary: An appropriate point on either the Professorial or Clinical Professorial salary scale.

To work for an employer that values and promotes equality of opportunity, visit www.cardiff.ac.uk/jobs telephone + 44 (0) 29 2087 4017 or email vacancies@cardiff.ac.uk for an application form quoting vacancy number 030.

Closing date: 7 March 2008.

THE HEALING FOUNDATION

U123254R

UNIVERSITY OF KONSTANZ

KoRS-CB, will commence its training program in April 2008. Thus, KoRS-CB invites applications for

Fellowships for Ph.D students

from highly motivated and enthusiastic students with a keen interest in interdisciplinary research and an excellent degree (Master or Diploma) in Biology, Chemistry, or related areas.

For details on the application procedure and further information on the research and training program of KoRS-CB, the participating Departments, and the University of Konstanz, please visit the KoRS-CB homepage at
www.chembiol.uni-konstanz.de

W121485R

UNIVERSITÄTSMEDIZIN GÖTTINGEN : UMG

DFG Research Center Molecular Physiology of the Brain (CMPB)

The Center for Molecular Physiology of the Brain (CMPB) invites applications for the position of

PROGRAM MANAGER (F/M)

Are you a creative and outgoing person who would be fascinated in helping scientists in the different disciplines of neuroscience to collaborate on projects that will take research from the "bench to the bedside"? Are you interested in public relations work, networking with scientists, Government officials, grant committees and members of the public to awaken interest and awareness for breaking-edge projects?

The CMPB unites a large number of specialized laboratories of several departments (Medicine, Biology, Physics) of the University of Göttingen, the Max Planck Institutes for Experimental Medicine and Biophysical Chemistry and the German Primate Center, all in Göttingen. Their interdisciplinary research activities are coordinated by a board of directors and its speaker. The CMPB is a scientific alliance for interdisciplinary research in molecular genetics, molecular biology, molecular physiology and nano-optics focusing on the development and application of new technologies to study ongoing molecular processes in neural cells and determine their specific contribution to brain functions, in both the normal and pathological states. The alliance aims at the translational implementation of innovative findings in clinical neurosciences. You, as program manager, would assist the speaker and program coordinators.

If you are the right person for the job you will hold a MD and/or PhD in the area of Neurosciences and have practical experience in this research area as well as in the management of national and international projects. You will exhibit outstanding communicative skills and team spirit, enjoy independence and have an excellent command of the English language. The initial contract would be for two years (with the likelihood of extension) at an attractive salary and public service advantages.

Women are especially encouraged to apply. Handicapped applicants with equal qualifications will be given preferential treatment.

Please send your written application with relevant documents within four weeks after the posting date of this announcement to **Prof. Dr. D.W. Richter, Department of Neuro- and Sensory Physiology, Humboldtallee 23, 37073 Goettingen, Germany,** Tel.: 0049-551/ 39 5911, E-Mail: d.richter@gwdg.de

W123092R

Vacancy Announcement

by the General Secretary of the Hungarian Academy of Sciences for

Positions of Director

in the following Research Institutes of the Hungarian Academy of Sciences:

Research Institute of Ecology and Botany
Institute of Biomolecular Chemistry (Chemical Research Centre)
Institute of Structural Chemistry (Chemical Research Centre)

The posts are available from 1st July 2008

Tasks of the director:
- directing and coordinating scientific activities at the Institute,
- maintaining and raising the Institute's scientific position and research role both nationally and internationally,
- accomplishing its scientific concepts as well as fulfilling its basic missions.

Requirements:
- scientific degree (Ph.D. or higher),
- prominent, internationally renowned results in at least one of the research fields of the Institute,
- experience in leadership of a research institute or relevant scientific unit,
- proficiency in English or another major language,
- proficiency in Hungarian.

Applications to be equipped with:
- a proposal by the applicant as to how he/she wishes to accomplish the scientific program of the Institute,
- a detailed professional CV,
- evidence of a scientific degree and languages spoken,
- a description of present job, position, and salary,
- a summary of professional achievements and career results,
- a full bibliography of scientific papers, studies, and books authored by the applicant,
- a declaration granting permission to reveal the contents of his/her application not only to the selection committee but also to HAS officials and administrators as well as to HAS's relevant scientific sections, and the research community of the Institute in question.

Each post is available for a maximum period of 5 years, or until the age of 70 years of the applicant. An official certificate of good conduct will be required for filling the position. You will find additional information on legal terms and provisons on the English home page of the Academy, in the News section:

http://www.mta.hu/index.php?id=406&type=0

Applications are to be sent both in hard copies and in electronic form (floppy or CD). They should be addressed to the General Secretary of the Hungarian Academy of Sciences and they have to reach the Legal and Administrative Department of the Secretariat of the Hungarian Academy of Sciences (H-1245 Budapest, P.O.Box 1000, Hungary) before 16:00 6th March, 2008 at the latest.

The names of successful applicants will be announced before 16th June, 2008.

For a description of the research profile of each Institute, you are invited to visit their respective home pages linked to:
http://www.mta.hu/index.php?id=676
Attila Meskó, General Secretary of HAS

W122124R

Faculty position in computational cancer genomics

The Cancer Genome Project at the Wellcome Trust Sanger Institute has led the way in the systematic analysis of cancer genomes and aims to continue this process using the new generation of sequencing technologies. We are seeking to appoint a Faculty member to lead a team in the analytical bioinformatics of cancer using genomic, mutational and expression data.

The successful individual will develop their own independent lines of research and will also work closely with the current members of the Cancer Genome Project. Faculty positions include funding to support a team commensurate with the seniority of investigator **(www.sanger.ac.uk/careers/faculty).**

For further information **or to register interest**, please see http://www.sanger.ac.uk/genetics/CGP/ and/or contact Mike Stratton, email mrs@sanger.ac.uk

Faculty applicants should send curriculum vitae, complete list of publications and details of three referees accompanied by:

- a summary of scientific achievements (approx 1-2 pages);
- an outline of research plans (approx 2-3 pages) indicating how these would link in with other Sanger programmes and infrastructure.

Please email to facultysearch@sanger.ac.uk or post to

Sancha Martin, Faculty Search Committee,
The Wellcome Trust Sanger Institute,
Wellcome Trust Genome Campus,
Hinxton, Cambridge CB10 1SA, UK

Application deadline: 8th February 2008
although positions will remain open until filled

www.sanger.ac.uk

Working towards equality through diversity

wellcome trust sanger institute

Genome Research Limited is a Registered Charity No.1021457

U123055R

www.embo.org

Promoting excellence in the molecular life sciences in Europe

EMBO support for young group leaders

Raise your PROFILE
\+ Widen your NETWORK
\+ Advance your RESEARCH
\+ Support your RESEARCH GROUP
= EMBO Young Investigator Programme

Application deadline
1 April 2008

Further details and eligibility
www.embo.org/yip

EMBO

W123228R

Senior Lecturer in Marine Zoology

School of Biological Sciences

£32,796 - £40,335

The School of Biological Sciences recruits across a wide range of undergraduate degree pathways including biology, forensic and marine biology and has an established reputation for research in environmental and biomolecular science (RAE5).

To maintain our balanced academic team, delivering high quality teaching and research, we are seeking to appoint a senior lecturer in marine zoology. Applications from those using molecular biological techniques for marine studies would be particularly welcome to enhance existing collaborations between areas of strength in the department.

Applications are invited from candidates with a strong record of research and/or knowledge transfer activity and a desire to contribute to undergraduate and postgraduate teaching programmes at all levels.

For an informal discussion, please contact Dr Matthew Guille, Head of School, email: matt.guille@port.ac.uk **Ref: ASCI 0054/N.**

To find out more about us and the roles we have on offer, visit www.port.ac.uk/vacancies and apply on-line. Alternatively, telephone 023 9284 3421. Please quote the reference number on all communications.

Closing date: 8th February 2008.

Personnel Services, University House,
Winston Churchill Avenue,
Portsmouth PO1 2UP.

Committed to equality of opportunity

INVESTOR IN PEOPLE

University of Portsmouth

Unleashing Potential

U123260R

nature chemistry

Associate Editors

The Nature Publishing Group is pleased to announce the launch of *Nature Chemistry* in 2009. Following the success of *Nature Materials, Nature Chemical Biology and Nature Physics,* and given the strength of the parent journal *Nature,* we fully expect *Nature Chemistry* to seize the commanding heights of the chemistry-publishing landscape.

Alongside the highest-quality original research, *Nature Chemistry* will cover news, commentary and analysis from and for the chemistry community, as well as striving to develop a voice that chemists care about.

As part of this exciting new publishing venture, we are now seeking three Associate Editors for *Nature Chemistry,* to be based in our London, Boston and Tokyo offices.

Applicants should have a PhD in a chemistry-related discipline, with demonstrable research achievements. Although postdoctoral experience is preferred (not required), emphasis will be placed on broadly trained applicants with a good knowledge of the chemistry community. Key elements of the position include the selection of manuscripts for publication, and commissioning, editing and writing other content for the journal. Candidates who wish to be considered for the role in our Japan office must demonstrate a good understanding of the East Asian research communities (in particular Japan, China and Korea) as well as being fluent in English and preferably an Asian language (Japanese, Chinese or Korean).

These are demanding and extremely stimulating roles, which call for a keen interest in the practice and communication of science. The successful candidates will, therefore, be dynamic, motivated and outgoing, and must possess excellent interpersonal skills. The salary and benefits, will be competitive, reflecting the critical importance and responsibilities of each position.

Applicants should send a CV (including their class of degree and a brief account of their research and other relevant experience), a News & View style piece (no more than 500 words) on a recent paper from the chemical literature, and a brief cover letter explaining their interest in the post, salary expectations, and indicating whether they wish to be considered for a position in London, Boston or Tokyo.

To apply please send your CV and covering letter, quoting reference number **NPG/LON/797** to Denise Pitter at londonrecruitment@macmillan.co.uk

The closing date for applications is Thursday 31st January 2008.

nature publishing group npg

IN121118R

School of Medical Sciences

Research Fellows

'Translational medicine: development of new biomarkers for CNS applications'

You will join a team of researchers studying transgenic CNS disease models (primarily for Alzheimer's disease and Schizophrenia). The project will focus on the establishment of translational biomarkers using behavioural, imaging (PET/CT) and electrophysiological methods, combined with standard cellular and histological procedures. These posts are available to complement our existing team working on this exciting project.

Post 1 (Ref: YMS287R): You should have experience in one or more of the following areas: animal behaviour, rodent disease models (CNS), in vivo imaging or EEG techniques. Experience in supervision and management of research projects would be an advantage.

Salary will be at the appropriate point on the Grade 6 scale (£27,857 – £33,262 per annum), with placement according to qualifications and experience.

Post 2 (Ref: YMS288R): You should ideally have experience in one or more of the following areas: molecular and biochemical assays such ELISAs, PCRs, Western blots, transgenic and viral transfection procedures, microscopy, immunocytochemistry.

Salary will be at the appropriate point on the Grade 6 scale (£27,857 – £29,553 per annum), with placement according to qualifications and experience.

Both posts are offered for a period of two years in the first instance.

Informal enquiries regarding the posts may be made to Dr Bettina Platt (tel: 01224 555741, e-mail: b.platt@abdn.ac.uk) or Professsor Gernot Riedel (tel: 01224 555758, e-mail: g.riedel@abdn.ac.uk).

Online application forms and further particulars are available from www.abdn.ac.uk/jobs. Alternatively, telephone (01224) 272727 (24-hour answering service) quoting the appropriate reference for an application pack.

Closing date: 15 February 2008.

Promoting Diversity and Equal Opportunities throughout the University

1495 UNIVERSITY OF ABERDEEN

U123224R

University of Glasgow

Faculty of Veterinary Medicine
Division of Animal Production & Public Health

Postdoctoral Research Assistant

£29,139 – £32,796

An immunologist or molecular parasitologist is required to study the interaction between Trichostrongylus retortaeformis and Graphidium strigosum in wild and laboratory rabbits.

For an application pack, please see our website at www.glasgow.ac.uk

Applications should be submitted to Brian O'Neil, Clinical Services Unit, Faculty of Veterinary Medicine, University of Glasgow, Bearsden Road, Glasgow, G61 1QH quoting Ref 13931/DPO/A3.

Closing date: 29 February 2008

The University is committed to equality of opportunity in employment.

www.glasgow.ac.uk Scottish University of the Year

U123265RM

Karakurum International University

Gilgit, Northern Areas, Pakistan

(The meeting place of the Himalayas, Karakoram and Hindu Kush)

Seeks Senior Faculty

Professors, Associate Professors, Assistant Professors for English, Mathematics, Education, Business Management, International Relations, Physics, Hotel Management and Mountain Tourism

KIU is an equal opportunity institution and females are encouraged to apply. It has 2,000 students (one third females).

Apply with bio-data and name of three referees to the Vice Chancellor at **vc@kiu.edu.pk** Salary range from Pak Rs.50,000 to 200,000 p.m. all inclusive according to rank (1 US$ = 60 Pak Rs.).

The Vice Chancellor will be in London shortly to meet interested individuals in UK.

For further details please visit **www.kiu.edu.pk**

RW123382R

VICE PRESIDENT DISCOVERY (RESEARCH) — BC CANCER AGENCY
ASSOCIATE DEAN, RESEARCH — UNIVERSITY OF BRITISH COLUMBIA

VANCOUVER, BRITISH COLUMBIA, CANADA

The BC Cancer Agency (BCCA) is committed to reducing the incidence of cancer, reducing the mortality from cancer, and improving the quality of life of those living with cancer. It provides a comprehensive cancer control program for the people of British Columbia by working with community partners to deliver a range of services, including prevention, early detection, diagnosis and treatment, research, education, supportive care, rehabilitation and palliative care. It operates four regional cancer centres in the Fraser Valley, Kelowna, Vancouver and Vancouver Island, a fifth centre is opening in Abbotsford in the summer, 2008, with a sixth centre scheduled to open in Prince George in 2012. In close association with the BC Cancer Agency's Research Centre, the regional centres conduct research into the causes and cures for cancer.

BCCA is an agency of the **Provincial Health Services Authority (PHSA)** which plans, manages and evaluates specialty and province-wide health care services across BC. ***PHSA embodies values that reflect a commitment to excellence. These include: Patients first • Best value • Results matter • Improvements through knowledge • Open to possibilities.***

Provincial Health Services Authority
Province-wide solutions. Better health.

The BC Cancer Agency is seeking a research leader of international profile with a record of scientific excellence to lead the 'discovery' research activities of the BC Cancer Agency. The appointment will also serve as Associate Dean, Research within the Faculty of Medicine at the University of British Columbia. The BC Cancer Agency is affiliated with the University of BC and co-hosts the development and conduct of academic oncology in BC. The position entails responsibility for visionary, strategic leadership and overall direction, coordination and oversight of 'discovery' cancer research at the BC Cancer Agency. The Discovery Research portfolio comprises 60 principal scientists and a total of 640 scientific and medical personnel organized within 10 research divisions. (Advanced Therapeutics, the Michael Smith Genome Sciences Centre, Cancer Control Research, Cancer Endocrinology, Cancer Genetics and Developmental Biology, Cancer Imaging, Medical Biophysics, the Terry Fox Laboratory, the Breast Cancer Research Chair program, and the Deeley Research Centre located in Victoria).

The BC Cancer Agency's Research Centre is one of the largest free-standing cancer research facilities in Canada, occupying 231,000 sq ft (Vancouver) and 18,000 sq ft (Victoria). Current research funding exceeds $60M per annum.

The University of British Columbia is Canada's third largest university and consistently ranks among the 40 best universities in the world. Primarily situated in Vancouver, UBC is a research-intensive university and has an economic impact of $4 billion to the provincial economy. The Faculty of Medicine at UBC, together with its partners including B.C.'s Health Authorities, provides innovative programs in the areas of health and life sciences through a province-wide delivery model. The Faculty teaches students at the undergraduate, graduate and postgraduate levels and generates more than $200 million in research funding each year. It is home to Canada's first distributed MD undergraduate program.

Applications are invited from individuals who hold PhD and/or MD qualifications. In addition, they should be eligible for appointment at the Associate or Full Professor level at University of British Columbia, and, if medically qualified and wishing to practice clinical medicine, hold or be eligible for Canadian specialist qualifications in the appropriate discipline and be eligible for licensure to practice medicine in BC. Key attributes of the successful applicant will include scientific excellence, innovation, collaborative relationships with local, national and international partners, promotion of 'team research' and the ability to lead a strategically focused program of excellence in scientific discovery.

A letter of application should be submitted with a current curriculum vitae to: **Stephanie Milliken - smilliken@telus.net** The closing date for applications is **March 10th, 2008.**

BC Cancer Agency
CARE + RESEARCH
An agency of the Provincial Health Services Authority

UBC

NW122387R

www.phsa.ca **www.bccancer.bc.ca**

UC DAVIS
UNIVERSITY OF CALIFORNIA

Foods for Health Initiative
University of California, Davis

OPEN FACULTY POSITIONS

The Foods for Health Institute (FFHI) at the University of California at Davis was created to foster programs at the intersection of food and health. It builds on UC Davis' great strengths and integrates across departments, schools and colleges to create multi-disciplinary research programs ranging from agricultural and food sciences to the clinical assessment of food components impact. We are currently recruiting eight Assistant Professor positions and a Director. Recruitments are near completion in phytonutrient biochemistry (Department of Plant Sciences) and food engineering (Departments of Food Science and Technology and Biological and Agricultural Engineering). Positions currently being recruited are:

- **Director, Foods for Health Institute:** Full Professor (Any related department). The Director will lead the initiative on both the main and health science campuses and is expected to be a highly accomplished scientist and administrator who can initiate, coordinate, and participate in research and education programs in the area of foods for health. For more information and to apply for the position go to http://recruitments.caes.ucdavis.edu/
- **Quantitative Systems Biologist:** Department of Biomedical Engineering. The appointee will emphasize development of quantitative methods and their applications to the integrated modeling of metabolism and underlying molecular mechanisms. For more information and to apply for the position contact Michael Savageau (masavageau@ucdavis.edu).
- **Biosensors:** Departments of Chemical Engineering and Material Science and Food Science and Technology. The appointee will demonstrate an ability to apply and analyze the application of analytical methods to complex food matrices. To apply for the position go to http://www.chms.ucdavis.edu/employment Interviews are currently being scheduled, however the position is open until filled.
- **Nutrition Toxicology and Metabolomics:** Departments of Nutrition and Environmental Toxicology. The appointee is expected to develop a strong research program in nutritional toxicology and metabolomics. Priority will be given to those with skills relevant to metabolic flux and pattern analysis and separation detection method (e.g. NMR, Mass spectrometry and/or high-throughput analysis platforms, etc.). To apply for the position go to http://ffh.recruitments.ucdavis.edu/
- **Metabolomics:** Departments of Nutrition and Food Science and Technology. The appointee is expected to develop a strong research program focusing on metabolic regulation (e.g. metabolic fluxes and patterns and integration of metabolic data with high information content). To apply for the position go to http://ffh.recruitments.ucdavis.edu/
- **Consumer Science:** Departments of Food Science and Technology and Nutrition. This position description is still under development and will be posted at a later date. Please visit the website http://ffhi.ucdavis.edu

All positions have the expectation of teaching and research and are academic year appointments. Some positions have the possibility of becoming fiscal-year term appointments, which hold a partial appointment in the Agricultural Experiment Station. To ensure consideration apply by February 29, 2008, however, all positions are open until filled.

UC Davis is an affirmative action/equal employment opportunity employer and is dedicated to recruiting a diverse faculty community. We welcome all qualified applicants to apply, including women, minorities, veterans, and individuals with disabilities.

NW122738R

FACULTY POSITION
ASSISTANT PROFESSOR

Genetics, Stem Cell or Systems Biology

Brigham and Women's Hospital
Harvard Medical School

The Genetics Division, Department of Medicine at Brigham and Women's Hospital and Harvard Medical School, seeks an outstanding Assistant Professor level scientist. Applicants should possess Ph.D., M.D., or M.D., Ph.D. degrees and have several years of relevant postdoctoral experience. Areas of interest are broad; these include human genetics, model organism genetics, systems level analyses of pathways involved in disease and development, stem cells, tissue and organ regeneration, and proteomics. The successful applicant will enjoy an outstanding competitive start-up package, excellent space and state-of-the-art facilities, and will be part of a division with diverse interests and expertise:

http://brighamandwomens.org/research/Genetics/

Please send, by 15th April, a CV, a 2-3 page description of research interests and plans, and 3 letters of recommendation to:

David Beier, M.D., Ph.D., Chair, Search Committee
c/o Suzanne Peterson, BWH Genetics Division
New Research Bldg., NRB 458D, Harvard Medical School
77 Avenue Louis Pasteur, Boston, MA 02115

Electronic submissions are preferred:
SPETERSON5@PARTNERS.ORG

BWH is an Equal Opportunity employer.

NW122566R

Senior Cancer Researcher

The Jackson Laboratory

Leading the search for tomorrow's cures

The Jackson Laboratory, a world-renowned mammalian genetics research institution and NCI-designated Cancer Center, is seeking an experienced **Senior Cancer Researcher** to assist in the expansion of the laboratory's Cancer Center Program. We are looking for a leader with a global view of cancer research, who can facilitate interdisciplinary and translational approaches while conducting a competitive, independently funded research program.

Candidates should have the following minimum qualifications:

- Extensive knowledge of cancer biology, a Ph.D. or M.D., and at least 10 years of independent research experience
- Strong scientific, organizational, management, and interpersonal communication skills
- The ability to interact effectively with internal scientists, external collaborators, as well as granting agencies
- Willingness and ability to recruit and mentor several new faculty members

We offer a unique scientific research environment, including excellent collaborative opportunities within our faculty, unparalleled mouse genomic resources, outstanding core scientific support services, highly successful pre- and postdoctoral training programs, a major scientific meeting center featuring courses and conferences centered on mouse models, and an idyllic research environment surrounded by Acadia National Park.

For information, go to: www.jax.org and www.jax.org/research/cancer_center.html

Applicants should send a curriculum vitae and a brief summary of their research to:

Robert Braun, Associate Director and Chair of Research
The Jackson Laboratory
600 Main Street, Bar Harbor, ME 04609
Interested individuals can also send an email to: **bob.braun@jax.org**

Applications should be received no later than **March 1, 2008.**

The Jackson Laboratory is an EOE/AA employer.

NW123013R

www.jax.org

JOHNS HOPKINS MEDICINE

The Department of Neurology of The Johns Hopkins University School of Medicine invites applications for a tenure-track faculty position for a

BASIC SCIENTIST or PHYSICIAN-SCIENTIST

participating in on-going laboratory based research in stroke, endothelial biology or CNS ischemic disease. Academic rank will be commensurate with qualifications and experience. The department has a strong focus on translational research in cerebrovascular disease with ample opportunities for collaborations with scientists from the Departments of Medicine, Anesthesia, Neuroscience and Neurosurgery.
The department features a breadth of inquiry encompassing molecular to clinical-patient oriented research and offers a supportive and collegial work environment.

Interested individuals should submit *curriculum vitae*, a summary of research interests and plans, and three letters of reference to:

Rafael H. Llinas, MD
Associate Professor of Neurology
The Johns Hopkins Bayview Medical Center, B122b
4940 Eastern Avenue
Baltimore Maryland. 21224
or electronically to rllinas@jhmi.edu

NW122752R

The Johns Hopkins University is an Affirmative Action/Equal Opportunity Employer

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Smithsonian Tropical Research Institute

IS SEARCHING FOR A RESEARCH SCIENTIST

The Smithsonian Tropical Research Institute (STRI), headquartered in the Republic of Panama, is seeking world-class scientists to establish research programs in any current field of marine or terrestrial research, working anywhere in the tropics. These fields include all biological sciences, anthropology, archaeology, paleontology, geology, and soils science. Ideally, research initiated by the successful applicants will complement existing programs (see **http://www.stri.org**). We are especially interested in research on vertebrate biology; anthropology and archaeology; paleontology; and climate change.

Panama and adjacent regions of tropical America are rich in terrestrial and marine habitats, archaeological sites and geological deposits. STRI maintains sophisticated research laboratories, a library, and support centers, in Panama City, as well as diverse facilities for field research throughout the tropics. A 100' vessel is available for marine and coastal research. STRI scientists maintain cooperative research programs with a world-wide network of institutions. Opportunities for mentoring young scientists are available through a vigorous fellowship program, and formal teaching is possible through programs with affiliated universities.

Applicants should have a Ph.D. degree and postdoctoral research experience in their fields. Interested candidates should submit a summary of research accomplishments and interests, curriculum vitae, five significant reprints, and the names and contact information of three potential referees.

Annual salary is commensurate with experience. Laboratory set-up and relocation expenses are provided. Housing and family education allowances may apply. Spanish-English bilingual education is available in some Panamanian schools, which follow international curricula and standards.

Review of applications will begin in April 2008. **Please send applications electronically to the Director of STRI, c/o Ms. Luz Latorraca, Office of Human Resources at: LatorraL@si.edu** Address inquiries to Dr. William Wcislo, Chair, Search Committee at: **WcisloW@si.edu**

STRI is an equal opportunity employer and appointments are made regardless of nationality.

NW123005R

Ecohydrology

The University of Vermont seeks to hire an outstanding Assistant or Associate Professor of Engineering, Mathematics, or Computer Science with emphasis on complex systems analysis in the context of Ecohydrology, the linkage between hydrological and ecological systems. The hire is part of a University focus on the environment and a College initiative in complex systems.

Qualifications for the position include an earned doctorate, a proven record of scholarly activities, and the ability to teach relevant graduate and undergraduate courses. Successful candidates will be expected to make significant and balanced contributions to research, teaching, and service, including the development of a nationally respected, externally funded research program.

Preference given to complete applications received before 2/20/2008. Submit CV, statement of research & teaching interests, and contact information for 3 references to www.uvmjobs.com (req#032225). AA/EO employer.

NW123129R

HEAD, DEPARTMENT OF GENETICS

NC State University, Raleigh, NC

The Department of Genetics at North Carolina State University invites nominations and applications for the position of Head of the Department. We seek an individual with outstanding leadership capabilities, proven academic and administrative experience, the vision to build and sustain programs at the forefront of genetics research, and a commitment to excellence. Candidates will be expected to maintain an externally-funded, high-impact research program.

The position announcement can be viewed in its entirety on the Department of Genetics Website: http://www.cals.ncsu.edu/genetics/ . Applicants should submit a letter of interest, curriculum vitae, statement of philosophy/vision as a Department Head to include research and teaching functions, and 3-5 representative publications electronically to http://ncsu.edu/jobs/ . Position Number is 01-64-0714.

Nominations and questions concerning this position should be sent to: **Damian Shea, Chair Genetics Head Search Committee, Department of Zoology, Campus Box 7617, NC State University, Raleigh, NC 27695-7617** or via email to **d_shea@ncsu.edu**

Review of applications will begin 15th February 2008 and continue until the position is filled.

AA/EOE. NC State University welcomes all persons without regard to sexual orientation.

NW122714R

UNIVERSITY OF MISSOURI-COLUMBIA

Research Associate

The Division of Pulmonary, Critical Care and Environmental Medicine at the University of Missouri-Columbia is seeking a research associate for a non-tenure track position. The successful candidate will be expected to participate and assist in the development of externally funded research programs. He/ she will have knowledge and practical experience in cell and molecular biology. Current projects being conducted require interest and experience in aerosol medicine, animal handling, and tissue culture. Interest, knowledge, and success in basic and translational research activity beyond specific area of interest are desirable. Qualifications for the position include Ph.D. or equivalent degree, at least 2 years of post-doctoral training, and an ability to independently maintain and mentor ongoing projects. Current projects in our lab include: Gene therapy in lung cancer cell lines, Nanoparticle application in lung cancer, in vitro and in vivo, Aerosolized delivery of various medications and gene therapy and Studies in mouse models of emphysema.

Interested applicants should submit a letter of application including a statement of research plans, curriculum vitae and contact information for three references. Please submit materials to **IMFaculty@health.missouri.edu**

UMC is an equal opportunity affirmative action employer and complies with the ADA act of 1990: Women and minorities are encouraged to apply. Questions and ADA accommodation needs may be addressed to Jessica Hosey, Human Resources Manager, 573-884-2825.

NW122741R

Visit the University of Missouri-Columbia's web site at http://mujobs.missouri.edu

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

Neuroscience Faculty Recruitment

The Department of Neuroscience at Columbia University Medical Center, as part of a University wide Neuroscience Initiative, is recruiting faculty concentrating on the analysis of neural circuitry through molecular, genetic, cellular electrophysiological and/or imaging approaches. We are particularly interested in individuals whose research program explores neural circuits in genetically tractable model systems and in the context of well-defined behaviors. We encourage applications for positions at the Assistant Professor level, but will also consider applications from more senior investigators for positions at the level of Associate or full Professor.

Columbia University currently has a world-renowned program in neurobiology and behavior and the Neuroscience Initiative aims to enhance interactions between basic and clinical neurosciences, and link the neurosciences to other scientific disciplines within the University. Faculty will be affiliated with the Department of Neuroscience, and there will be opportunities for strong ties with scientific departments and programs on the Morningside Heights campus.

Applications for this round of recruitment are requested by March 1, 2008. A C.V., cover letter including statement of interests, and three letters of reference under separate cover should be emailed care of David Leyden, dgl2102@columbia.edu. In addition, please mail a hard copy of these documents to:

Chair, Neuroscience Search Committee
c/o: David Leyden
Columbia University
Hammer Health Sciences Center
Room 2-205G
701 W 168th Street, New York N.Y. 10032

Columbia University takes affirmative action to ensure equal opportunity

NW122261R

DTU

PROFESSOR

(WITH SPECIAL RESPONSIBILITIES)

In order to specifically strengthen the efforts of the Department of Chemical and Biochemical Engineering to develop a leading position in the field of chemical engineering aspects of biocatalytic food ingredient processing, the department has with a grant from Danisco decided to establish the five year "Danisco-LMC Professor Position".

Further information from Professor Kim Dam-Johansen, Head of Dept., Department of Chemical and Biochemical Engineering, kdj@kt.dtu.dk

The full text of the announcement is available on the home page of DTU.

Application deadline: 8th Feburary 2008 at 12.00.

The Technical University of Denmark is one of the leading technical research and educational institutions in Northern Europe with 6.200 students, 4.500 employees and a yearly turnover of DKK 3.1 billion.

As of January 1, 2007 DTU has merged with the Danish Institute for Food and Veterinary Research, Risø National Laboratory, the Danish Institute for Fisheries Research, the Danish National Space Centre and the Danish Transport Research Institute.

Further details www.dtu.dk/vacancy

W123053R

MANCHESTER 1824

The University of Manchester

www.manchester.ac.uk/jobs

Faculty of Life Sciences

Postdoctoral Research Associate in Developmental Biology

Ref: LS/012/08

£26,666 - £32,796 p.a.

The post is based in the laboratory of Professor Nancy Papalopulu, which is part of a growing and dynamic group of Developmental Biologists. The project will investigate the mechanisms of cell polarity and spindle orientation in Xenopus. Experience in Xenopus embryology, imaging techniques and cell biology is required for this post. See Strauss et al., 2006, Development, 133-3883-3893.

Further information can be found at: www.manchester.ac.uk/developmentalbiology

A relevant PhD and at least one recent first author publication in an international journal is required.

Funded by the Wellcome Trust, this position is tenable immediately until 31 August 2010.

Closing date: 14 February 2008.

Research Assistant in Developmental Biology

Ref: LS/011/08

£22,332 p.a.

A Research Assistant position is available to assist in a project investigating the origin of neural stem cells in Xenopus and to provide general laboratory support. You will have a first degree (or equivalent) in a biological science and excellent organisational skills. Some experience in molecular and cell biology particularly in the generation and use of antibodies would be an advantage.

Funded by the BBSRC, this position is tenable from April 2008 until 15 April 2010.

Application forms and further particulars for the above two posts are available from our website or by contacting Rachel Abbott on +44 (0) 161 275 5360 or rachel.abbott@manchester.ac.uk quoting the reference number.

Closing date: 14 February 2008.

Research Assistant in Stem Cell Biology

£22,332 p.a. **Ref: LS/010/08**

This position is based within the research group of Professor Cay Kielty. The post involves analysis of how platelet-derived growth factors and vascular endothelial growth factor bind platelet-derived growth factor receptors and regulate their signalling in adult stem cells. This study is part of a major ongoing programme on adult mesenchymal stem cell biology.

You will have a good degree in a relevant area of biological science, and appropriate experience in cell and molecular biology research is highly desirable.

Funded by the MRC, this position is tenable from 1 March 2008 until 30 November 2010.

Informal enquiries can be addressed to Professor Cay Kielty on: +44 (0) 161 275 5082 or cay.kielty@manchester.ac.uk

Application forms and further particulars are available from our website or by contacting +44 (0) 161 275 8836 or lifesciences-hr@manchester.ac.uk quoting the reference number.

Closing date: 7 February 2008.

The University will actively foster a culture of inclusion and diversity and will seek to achieve true equality of opportunity for all members of its community.

U123253R

Marie Curie Research Institute

Oxted, Surrey, RH8 0TL, UK

Postdoctoral Research Fellow Position In Cell Cycle Control

Salary range £27,062 - £33,282

Applications are invited for a Postdoctoral Fellowship in the MCRI Cell Cycle Control Laboratory, headed by Dr. Hiro Yamano, who studies how the ubiquitin-mediated proteolysis controls cell cycle. The project will focus on mechanisms of the APC/C (anaphase-promoting complex/cyclosome) dependent ubiquitylation and proteolysis using frog egg extracts and fission yeast (Kimata et al., Dev. Cell, in press). Further information on the institute and the laboratory can be found on our website: http://www.mcri.ac.uk/research/cellcycle.asp

Applicants with experience in one or more aspects of molecular biology, biochemistry, genetics or cell biology will therefore be an advantage. However, we will consider highly motivated and enthusiastic individuals, who tackle our research projects. We offer a very competitive salary, which will depend upon qualifications and skills. The institute is located in an extremely pleasant, rural environment in Oxted, Surrey, with easy access to the London area.

Candidates should submit a full CV, with the names and addresses of three referees and a brief description on intent of research, to Dr. Hiro Yamano, Cell Cycle Control Laboratory, Marie Curie Research Institute, The Chart, Oxted, Surrey RH8 0TL, UK.

E-mail: h.yamano@mcri.ac.uk

The Marie Curie Research Institute is committed to equal opportunities in employment

U122569R

POST DOCTORAL FELLOWSHIP

MRC National Institute for Medical Research

Situated in Mill Hill, North West London, NIMR is the largest MRC institute, supporting some 70 research groups and 500 bench scientists. The Institute provides excellent training for researchers in a multi-disciplinary environment and is equipped with state of the art facilities. **http://www.nimr.mrc.ac.uk/employment/**

Division of Molecular Neuroendocrinology **Ref:NIMR08/024**

Molecular regulation of exocytosis in human endothelial cells

We are offering a three year Career Development Fellowship. The project will focus on the identification of molecular components regulating exocytosis of secretory organelles in human endothelial cells. Exocytosis of two distinct organelle populations will be studied; the Weibel Palade body, and a constitutive secretory organelle. The project will involve a combination of molecular, biochemical and biophysical approaches to study directly the roles of identified molecular elements in secretory vesicle transport, docking and fusion using live cell imaging including confocal, wide field and total internal reflection fluorescence microscopy.

It is essential that you have A Ph.D. or equivalent in biochemistry, molecular/cellular biology/biophysics. You should have experience of use of recombinant DNA techniques to make mammalian expression vectors, knowledge of working with mammalian cells in culture. And experience of basic biochemical techniques and general interest in mammalian secretory pathway.

Informal enquires can be made to Dr Tom Carter **tcarter@nimr.mrc.ac.uk** **http://www.nimr.mrc.ac.uk/molneuroendo/carter/**

Salary is from £26,405 to £32,357 per annum inclusive of Location Allowance. MRC final salary Pension Scheme is available.

Applications for this role must now be made online at **http://jobs.mrc.ac.uk.** If you do not have internet access or you experience technical difficulties please call 01793 301157.

The closing date is 21 February 2008.

The MRC is an Equal Opportunities Employer

U123249R

University of Oxford

Nuffield Department of Clinical Medicine (ORCRB) The Structural Genomics Consortium (SGC)

Principal Investigator – Chemical Biology

Salary scale for University Staff Grade 10:

£45,397 - £52,628 p.a.

The Structural Genomics Consortium (SGC), established in 2003, is an international collaboration of British, Canadian & Swedish scientists whose primary objective is to determine the 3-dimensional structures of human and malarial proteins by X-ray crystallography and NMR spectroscopy and release them into the public domain (public databases). The SGC has now built the infrastructure and technology platforms to determine the structures of 200 proteins per year. Currently, the output from the SGC (globally) is ~25% of all novel human protein structures deposited in the protein data bank. SGC-Oxford has to date deposited ~200 novel human protein structures (and ~250 in total, including non-counted follow-up structures) within its main focus areas, phosphorylation dependent signaling, metabolic enzymes as well as integral membrane protein signaling. The consortium, which is funded by the Wellcome Trust, a consortium of Canadian and Swedish funding agencies as well as GlaxoSmithKline, is one of the largest structural proteomics projects in the world with ~170 staff in total.

SGC Oxford is currently located in the Botnar Research Building at the Nuffield Orthopaedic Centre. It is anticipated that the Chemical Biology Group will consist of 5-6 scientists and will work in close collaboration with other research groups at SGC Oxford as well as with our other sites (in Toronto and Stockholm). Further details can be found on our website: http://www.sgc.ox.ac.uk/

We are seeking a highly skilled, motivated and experienced scientist, with a PhD or equivalent in Chemistry, Biochemistry, Biophysics or related areas, to lead and provide expertise in the identification and validation of interaction partners (chemical modulators of function, natural ligands as well as protein domains) focused on specific human protein targets and families which can be used in structural studies and to provide chemical probes for selected target proteins. In addition, the research of the group should provide insights for structure-function relationships of inhibitors. Key goals are to build a target-focused compound collection, to implement and optimise efficient screening infrastructure and methodologies, as well as to establish and develop further validation assays. You will have an international reputation in science and technology and an extensive publication or development record in a relevant field. You will also have experience in project management, including screening and functional validation activities, team leadership and supervisory experience.

The post is available immediately to the 30 June 2010 in the first instance, with a view to extending it to 30 June 2011 when full funding for the SGC Phase II activities has been fully awarded. An application form and a job description are available from the Personnel Officer, ORCRB, University of Oxford, Old Road Campus, Off Roosevelt Drive, Headington, Oxford OX3 7DQ (tel. (01865) 289453), e-mail: orcrb.personnel@ndm.ox.ac.uk Please quote the reference HC-08-003-SGC. The closing date for applications is 15 February 2008.

As an Equal Opportunity employer, we positively encourage applications from people of all backgrounds

U123237RM

www.ox.ac.uk/jobs

School of the Environment & Natural Resources
Centre for Evidence - Based Conservation

Research Officer

Starting Salary: £21,478 - £24,839 (on R&A Grade 1A) p.a.

Using systematic review methodology to establish an evidence base for implementing climate change strategy.

Applications are invited for the above post to work on this NERC funded project led by Professor Andrew Pullin in the Centre for Evidence-Based Conservation (www.cebc.bangor.ac.uk), in collaboration with the Environment Agency Wales (EAW).

The post-holder will be responsible for undertaking systematic reviews of the available research evidence relating to a series of issues of current importance in environmental management, identified by the EAW in their Climate Change Strategy Implementation Plan. Also, for dissemination of the findings of the reviews using a variety of communication media, including www.environmentalevidence.org, to different audiences in this field. They will promote effective use of outputs of systematic reviews, testing their utility for informing the decision-making process and establishing research needs within EAW.

The successful applicant will possess, or be studying towards, a PhD in a relevant subject. They will have experience of literature searching of scientific information both through electronic media and manual methods, excellent statistical skills, experience of writing scientific papers, preferably in an environment-related subject, good computer literacy including familiarity with use of spreadsheets and good communication and team working skills. They will also possess a strong interest in environment management and conservation and a good understanding of the environmental sector. This post is for a period of 3 years and the successful candidate will be expected to start 1st April, 2008 or as soon as possible thereafter.

Application forms and further particulars should be obtained by contacting Human Resources, Bangor University; tel: (01248) 382926/388132; e-mail: personnel@bangor.ac.uk; web: www.bangor.ac.uk

Please quote reference number 08-7/97 when applying.

Closing date for applications: Friday 15th February 2008. Interviews will be held either the last week in February or first week in March 2008.

Informal enquiries can be made by contacting Professor Andrew S. Pullin, tel: (01248) 382444; e-mail: a.s.pullin@bangor.ac.uk.

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U123243R

Vetsuisse-Faculty, University of Bern

The position of an

Associate Professor (Extraordinarius) in Veterinary Bacteriology

specialized in the field of molecular epidemiology, infectiology and antibiotic resistance of animal diseases and zoonoses is available at the Vetsuisse-Faculty of the University of Bern.

Requirements
PhD and/or habilitation in the field of molecular epidemiological Veterinary Bacteriology and internationally renowned research activity.

Languages
English, German and/or French.

More detailed information can be obtained from the Dean's office.
Phone: +41 31 6312520, Email: ines.fischer@vetmed.unibe.ch

Applicants should submit their CV including a list of publications as well as a brief outline of their concept for research and teaching no later than April 30, 2008 by mail and electronically to

Dean's office
Prof. Dr. A. Zurbriggen
Vetsuisse-Faculty, University of Bern
Länggass-Strasse 120, Postfach
CH-3001 Berne/Switzerland

u^b UNIVERSITÄT BERN

Universität Bern | Universität Zürich
vetsuisse-fakultät

W122412R

John Innes Centre

Independent Research Fellowships

The John Innes Centre (JIC), Norwich, UK is a world leading centre of excellence in plant and microbial sciences based on the Norwich Research Park. We are inviting applications from outstanding researchers who either hold, or wish to apply for Independent Research Fellowships, to attend a Conference at the JIC on 2/3 June 2008. At the meeting you will be able to present a talk about your proposed area of research and to discuss your proposals, the development of your group and your future career plans in depth with senior JIC Scientists.

After the Conference we will select and mentor outstanding candidates in writing. Fellowship applications and/or offer the opportunity to move existing Fellowships to the JIC.

Further details and particulars can be found at http://www.jic.ac.uk/corporate/opportunities/vacancies/fellows.htm

Please e-mail a 2-page summary of your research plan, a copy of your CV and arrange for these letters of recommendation to be emailed to dawn.barrett@bbsrc.ac.uk by Friday 18th April 2008.

The John Innes Centre is a registered charity (No223852) grant-aidedby the Biotechnology and Biological Sciences Research Council and is an Equal Opportunities Employer.

U123172R

University of Oxford

Sir William Dunn School of Pathology

Post-Doctoral Research Assistant

£26,666 - £32,796 p.a.

We are seeking to appoint a post-doctoral Research Associate (Grade 7) in a Wellcome Trust funded laboratory with research interests in mammalian developmental biology located at the Sir William Dunn School of Pathology.

Under the direction of Professor Elizabeth Robertson FRS, WT-PRF, the programme aims to understand the molecular signals responsible for cell growth and differentiation in the early mammalian embryo. We have used genetic approaches to investigate the roles of TGFß growth factor signalling pathways in patterning the early body axis and in organogenesis. We are particularly interested in understanding the molecular mechanism by which specific transcription factors regulate the expansion of diverse progenitor cell populations in the embryo. Candidates with experience in targeted mutagenesis, cell imaging, flow cytometry, microarray, CHIP and/or proteomics approaches would be especially welcome. We are looking for a well trained and motivated scientist who is able to work independently and contribute conceptually to the overall research programme. Good organisational and communication skills are essential. This post is funded for one year in the first instance with the possibility of extension thereafter.

Please send your letter of application with full CV and the names and addresses of two referees to: Administrator, Sir William Dunn School of Pathology. University of Oxford, South Parks Road or e-mail: administration@path.ox.ac.uk by the 22 February 2008. Please quote reference LR/08/002.

The University and Jesus College are Equal Opportunity Employers. We positively encourage applications from people of all backgrounds

U123247RM

www.ox.ac.uk/jobs

Division of Maternal and Child Health Sciences
College of Medicine, Dentistry & Nursing

POSTDOCTORAL SCIENTIST/RESEARCH ASSISTANT

Temporary six month, post in the first instance

£23,692 – £32,796

A scientist is required to join a small and newly established research team in reproductive medicine responsible to Professor Christopher Barratt. Working closely with the assisted conception unit (IVF) the post involves examination of human sperm function in particular activation by fluids present in the human reproductive tract. The candidate should be familiar with basic cell and molecular biology techniques e.g. Western blotting. If necessary, the candidate will be taught human sperm function assays. Ideally applicants should have a BSc and a PhD, however, those graduates with research experience will be considered.

Informal enquires to Christopher Barratt c.barratt@dundee.ac.uk. We regret we cannot accept applications by e-mail.

Further details and an application pack are available from our website www.jobs.dundee.ac.uk Alternatively, contact Personnel Services, University of Dundee, Dundee DD1 4HN, tel: (01382) 384817 (answering machine). Please quote reference number MD/2030/N. Closing date: 7 February 2008.

As part of the recruitment process, the University requires that a Disclosure Scotland check is undertaken for this position.

The University of Dundee is committed to equal opportunities and welcomes applications from all sections of the community.

U123233R

www.dundee.ac.uk/jobs

UNIVERSITY OF DUNDEE

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The Royal Society's Medals and Awards are an important part of our work in recognising excellence in science across the disciplines and exist to reward those who have made outstanding achievements.

The deadline for all 2008 nominations is **25 February 2008**.

Prize lectures awarded in 2008 will be delivered in 2009.

Biological sciences
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Physical sciences
Royal Medal, Davy Medal, Hughes Medal, Leverhulme Medal, Rumford Medal, Bakerian Lecture and Clifford Paterson Lecture.

Women in science
Rosalind Franklin Award.

Science communication
Michael Faraday Lecture and Royal Society Kohn Award.

Philosophy and history of science
Wilkins-Bernal-Medawar Lecture.

International
Royal Society and Académie des sciences Microsoft European Science Award and Royal Society Pfizer Award.

Full details of all awards can be found at:

royalsociety.org/awards

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U121035A

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Call for nominations will close March 1, 2008.

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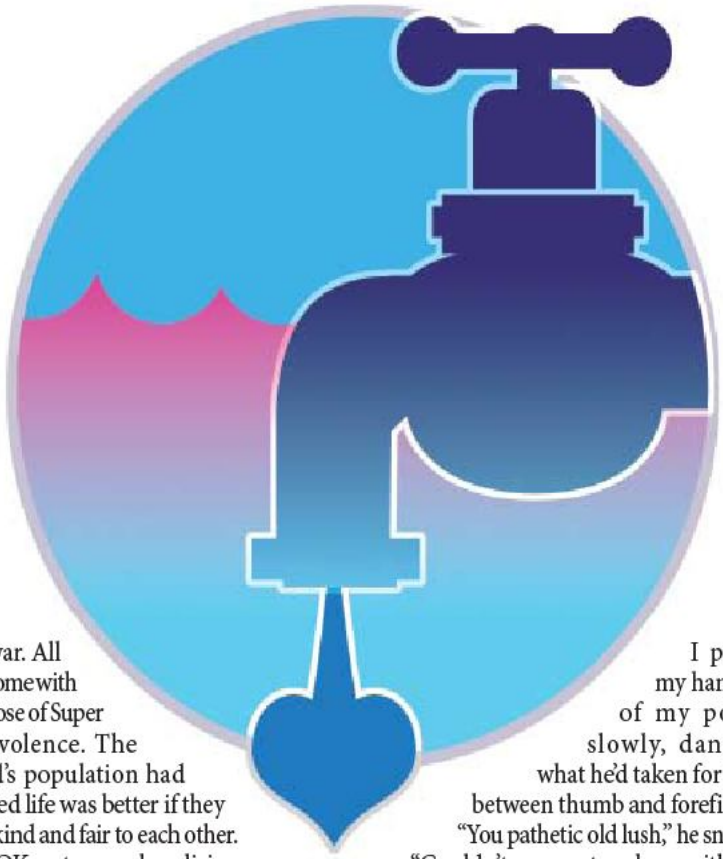
SuperB

Raise a glass to world domination.

Janet Wright



JACEY

The SuperB virus spread like wildfire — which was funny, really, because we'd put it in the water.

Forty years I spent perfecting it, while people were laughing behind their hands. Poor drunken old Dr J, mad as a bat in her dusty old lab, doing her useless antiviral work. Or so they thought. They're not laughing at me now, are they?

Once we'd perfected SuperB, my dedicated little group scattered it around the globe. The virus was sprinkled into the headwaters of the Nile, dripped into remote springs and slipped directly into the water glass of a particularly irritating politician. Released into reservoirs, SuperB was lifted into the clouds to fall on fields and gardens. It was sipped from bottles by yuppies and from streams by goat-herders. And it was so powerful that a few drops of rain turned an entire well into a SuperB reservoir.

The effects became noticeable very suddenly and, crucially, across the whole world at the same time. Crowds in the streets; emergency government debates; a few hopeless attempts to mobilize armies. But against what?

My beautiful transgenic virus, after so much patient fine-tuning, targeted just the right few cells in the brain. It did its work invisibly, unstoppably. Meanwhile, who noticed mad old Dr J doing a triumphant jig in the moonlight?

Then something started going wrong. And I realized I hadn't seen one of my group for a while, that icy young Mark who'd talked about the need for an antidote, 'just in case'.

Two of my dear little helpers visited Mark's girlfriend. Some gentle persuasion got all the information we needed, including an address.

Mark was just the sort of messianic idiot to build his secret lab on a mountaintop. Smiling passers-by took my arm to help me struggle up to the peak. Others guided me to a shady rock where I could sit while my vision cleared.

There I drank some gin from my hip-flask and rested for a while, looking down at the world I had shaped. My followers, a group of renegade Buddhists, had told their willing converts that SuperB was the cure for everything, and they had believed it.

Why shouldn't they? Most of the world's problems were caused by greed, self-pity or aggression, and what remained could soon be solved without the distractions of crime and war. All overcome with one dose of Super Benevolence. The world's population had realized life was better if they were kind and fair to each other. Well, OK, not so much realizing as being unable to do anything else. My plan for world peace coming to fruition. And now that little creep was messing it up.

Breathing heavily, I pushed the lab door open. Mark was facing me from the other side of the high lab bench, grinning. "Glad you could drop in," he said jovially. "I'm sure you'd like to see some of the improvements I've made."

"You call those improvements, do you?" I said. "Violence, terror, crime? I'm disappointed." Even as I spoke I was aware how feeble it sounded to him.

"Disappointed?" he yelled, dropping the friendly act. "I'm disappointed! Your reputation as a tough, hard-drinking loner — what a joke! I thought you were planning to take over the world."

"We have taken it over," I pointed out. He shook it off: "You're the smuggest do-gooder I ever met! You make me sick." His eyes were strangely bright. Could I really see tears sparkling at their brims? If he was losing his grip, I had a chance.

"Poor boy," I cooed. "Your so-called antidote lasts only a few days. Bank robbers are giving back what they stole, with interest. Those gunmen who broke into the Vatican have taken holy orders."

"I'm taking the antidote every day while I work on it," he said. "And what a pity you can't stop me, Dr Nicey-Nice. You can pull out that gun, but you can't shoot me, can you? Because you haven't taken my antidote."

I pulled my hand out of my pocket slowly, dangling what he'd taken for a gun between thumb and forefinger.

"You pathetic old lush," he sneered. "Couldn't even get up here without a drink to steady your nerves."

I went on smiling, looking him in the eye, the gin bottle swinging from my fingers in a hypnotic rhythm. It finally dawned on him.

"You've never drunk any water," he croaked. "You haven't got the SuperB virus." The next two thoughts — "So you can shoot me" and "Now you really have pulled out a gun" — hit him faster. He dived for the door, but you don't have to be young and quick to hit someone from that distance without a lab bench in the way.

He went down dramatically, sweeping a benchful of phials with him. I emptied the bottle into his mouth before he smashed it with his last conscious movement.

I put away the tranquillizer gun and had a couple of drinks from my hip-flask before Mark got up, tears running down his face.

"May I clean this place up?" he whispered. I knew the urge was overwhelming. Of course, I lifted my feet helpfully as he ran the vacuum cleaner around. Even I couldn't survive on gin alone: I was as infected by Super Benevolence as everyone else on the planet.

But I made sure he disposed very carefully of the broken bottle, which had contained my hastily concocted virus update. It had some odd side effects, including the compulsive housework. And I was damned if I wanted to risk catching that. ■

Janet Wright has written nine books about health, and says science has proved that dancing is better for you than housework.

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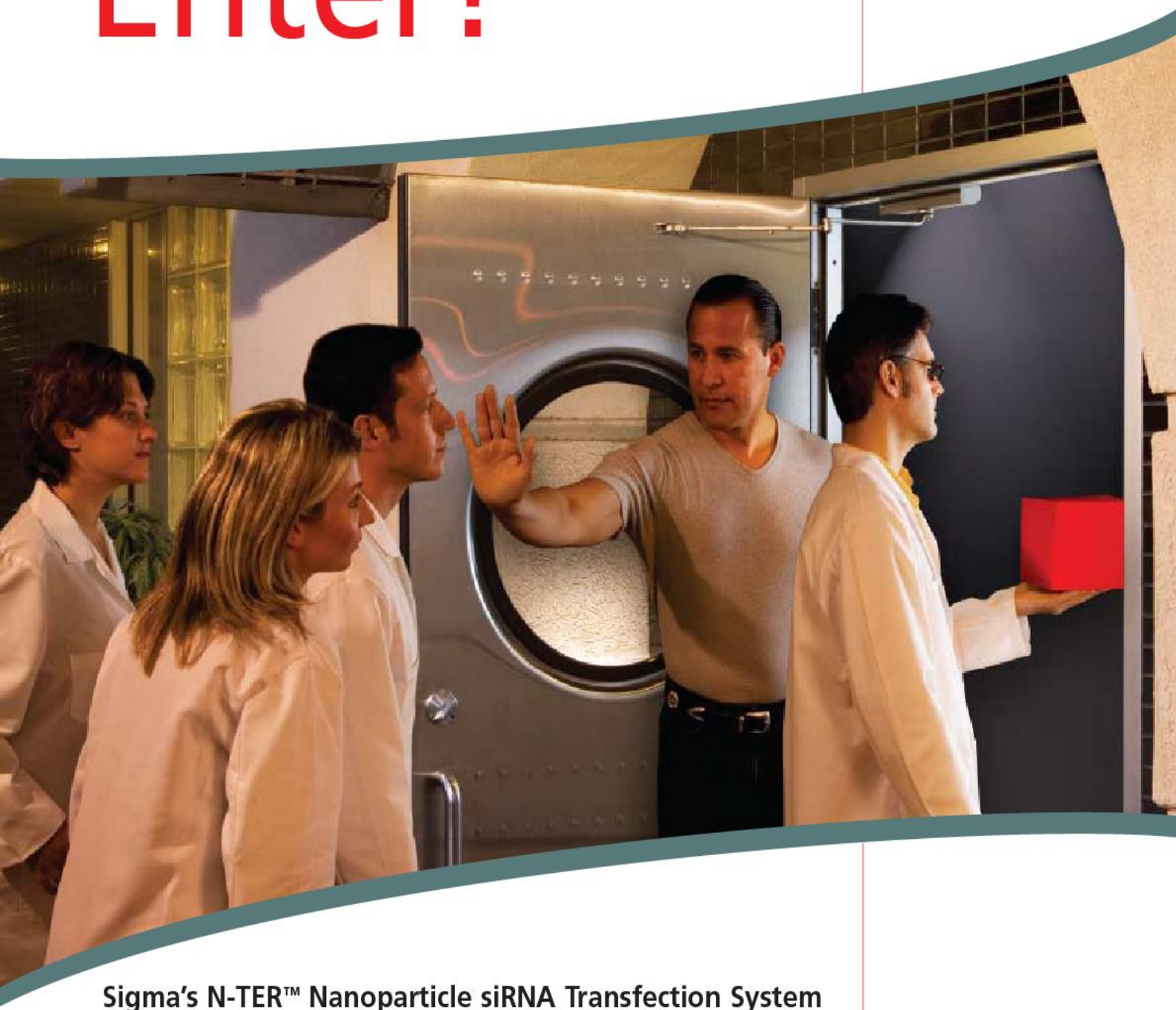

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